PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 March 2001 (15.03.01)	
International application No. PCT/KR00/00713	**Applicant's or agent's file reference** 0FPO-05-03
International filing date (day/month/year) 03 July 2000 (03.07.00)	**Priority date** (day/month/year) 05 July 1999 (05.07.99)
Applicant JUNG, Kyeong-Eun et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 February 2001 (02.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
LEE, Won Hee

8th Fl., Sungji Heights II, 642-16, Yeoksam-dong, Kangnam-gu, Seoul 135-080, Republic of Korea

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)* 24 NOVEMBER 2001 (24.11.2001)

Applicant's or agent's file reference
0FPO-05-03

IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/months/year)*
PCT/KR00/00713	03 JULY 2000 (03.07.2000)	05 JULY 1999 (05.07.1999)

Applicant

DONGBU HANNONG CHEMICAL CO., LTD. et al

1. The applicant is hereby notified that International Preliminary Examining Authority transmits here with the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report(but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details in the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea	COMMISSIONER
Facsimile No. 82-42-472-7140	Telephone No. 82-42-481-5210

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference 0FPO-05-03	**FOR FURTHER ACTION**	SeeNotificationofTransmittalofInternationalPreliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR00/00713**	International filing date *(day/month/year)* 03 JULY 2000 (03.07.2000)	Priority date *(day/month/year)* 05 JULY 1999 (05.07.1999)

International Patent Classification (IPC) or national classification and IPC

IPC7 C07H 21/00

Applicant

DONGBU HANNONG CHEMICAL CO., LTD. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
02 FEBRUARY 2001 (02.02.2001)	19 NOVEMBER 2001 (19.11.2001)
Name and mailing address of the IPEA/KR Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LIM, Hea Joon Telephone No. 82-42-481-5590

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR00/00713

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[ ] the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the claims:
pages _____, as originally filed
pages _____, as amended (together with any statment) under Article 19
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the drawings:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

[ ] the sequence listing part of the description:
pages _____, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application(under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained inthe international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages _____

[ ] the claims, Nos. _____

[ ] the drawings, sheet _____

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR00/00713

V. Reasoned statement under Article 35(2) with regard to novelty, inventiv step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO
Inventive step (IS)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

1) The following document have been considered for the purpose of this report:

D1=US 5,623,070 (Apr. 22, 1997)

D2=US 5,386,023 (Jan. 31, 1995)

2) Novelty

Claims 1-4, 5-7, 8, 9, 10-12, 13-14 relate to a nucleotide monomer in which a five-membered ribose is substituted with a six-membered azarsugar, antisense oligomers, the process for preparation thereof. The closest documents related to this invention is document D1 and D2.

Even the document D1 and D2 disclosed antisense drugs, oligomers with modified sugar in the oligonuceltides, the sugar structure of the document D1 and D2 were different from those of this invetion. Document D1 and D2 discloses modified nucleotide monomer which substitutes the natural sugar with the five membered ribose ring. E

Since claims 1-4, 5-7, 8, 9, 10-12, 13-14 in this invention discloses six-membered azarsugar, different from five membered ribose ring in document D1 and D2, those claims are considered to be novel.

3) Inventive Step

Claims 1-4, 5-7, 8, 9, 10-12, 13-14 relate to a nucleotide monomer in which a five-membered ribose is substituted with a six-membered azarsugar, antisense oligomers, the process for preparation thereof. Document D1 and D2 discloses nucleotide base with the five membered ribose ring.

The major difference of this invention and document D1 and D2 is sugar ring structure, which contributes better functional groups for this invention. The six-membered azarsugar are useful for developing antisense drugs due to high binding affinity to mRNA and good membrane permeability and improved resistance to nuclease. The high binding affinity is shown as high Tm value in this invention, which is important technical effects for this invention.

Therefore, claims 1-4, 5-7, 8, 9, 10-12, 13-14 in this invention appear to involve an inventive step.

4) Industrial applicability

The subject matter of claims 1-4, 5-7, 8, 9, 10-12, 13-14 is considered to be industrially applicable.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

LEE, Won Hee

8th Fl., Sungji Heights II, 642-16, Yeoksam-dong, Kangnam-gu, Seoul 135-080, Republic of Korea

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 0FPO-05-03	Date of mailing *(day/month/year)* 29 NOVEMBER 2000 (29.11.2000) **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. **PCT/KR00/00713**	Intrenational filing date *(day/month/year)* 03 JULY 2000 (03.07.2000)

Applicant

DONGBU HANNONG CHEMICAL CO., LTD. et al

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsilime No.: (41-22) 740.14.35

For more detailed instructions, see the n[illegible] the accompanying sheet.

2. [ ] The applicant is hereby notified that no inte[illegible]rch report will be established and that [illegible]claration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
[ ] the protest together with the decision theron has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision theron to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, nust reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively. before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/KR Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer COMMISSIONER Telephone No. 82-42-481-5131

Form PCT/ISA/220 (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
0FPO-05-03		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/KR00/00713	03 JULY 2000 (03.07.2000)	05 JULY 1999 (05.07.1999)
Applicant		
DONGBU HANNONG CHEMICAL CO., LTD. et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
      ☐ contained in the international application in written form.
      ☒ filed together with the international application in computer readable form.
      ☐ furnished subsequently to this Authority in written form.
      ☐ furnished subsequently to this Authority in computer readable form.
      ☐ the statement that the subsequenlty furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
      ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawing** to be published with the abstract is Figure No. _____
   ☐ as suggested by the applicant.
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.
   ☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00713

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07H 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC7 C07H 21/00

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the internationl search (name of data base and, where practicable, search trerms used)
STN caslink database structure search, IBM patent database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9932481 A1(Alcon Laboratories Inc.) 1 Jul. 1999 (7.01. 1999)	1-8
A	US 5623070 A (Isis Pharmaceuticals Inc.) 22 Apr. 1997 (4. 22. 1997)	1-8
A	US 5386023 A (Isis Pharmaceuticals Inc.) 31. Jan. 1995 (1. 01. 1995)	1-8
P	US 5965721 A (Isis Pharmaceuticals Inc.) 12. Oct. 1999 (10. 12. 1999)	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 NOVEMBER 2000 (22.11.2000)	28 NOVEMBER 2000 (28.11.2000)
Name and mailing address of the ISA/KR Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LIM, Hea Joon Telephone No. 82-42-481-5590

Form PCT/ISA/210 (second sheet) (July 1998)

NK

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COPY FOR IB

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Artcle 36 and Rule 70)

Applicant's or agent's file reference 0FPO-05-03	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. **PCT/KR00/00713**	International filing date *(day/month/year)* 03 JULY 2000 (03.07.2000)	Priority date *(day/month/year)* 05 JULY 1999 (05.07.1999)

International Patent Classification (IPC) or national classification and IPC

IPC7 C07H 21/00

Applicant

DONGBU HANNONG CHEMICAL CO., LTD. et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demamd	Date of completion of this report
02 FEBRUARY 2001 (02.02.2001)	19 NOVEMBER 2001 (19.11.2001)
Name and mailing address of the IPEA/KR Korean Intellectual Property Office Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon Metropolitan City 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer LIM, Hea Joon Telephone No. 82-42-481-5590

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR00/00713

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[ ] the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statment) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[ ] the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application(under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide** and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained inthe international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disc losure in the international applicationas as filed has been furinshed.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages _____

[ ] the claims, Nos. _____

[ ] the drawings, sheet _____

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box(Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed." and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International aplication No.
PCT/KR00/00713

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO
Inventive step (IS)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-4, 5-7, 8, 9, 10-12, 13-14	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

1) The following document have been considered for the purpose of this report:

D1=US 5,623,070 (Apr. 22, 1997)

D2=US 5,386,023 (Jan. 31, 1995)

2) Novelty

Claims 1-4, 5-7, 8, 9, 10-12, 13-14 relate to a nucleotide monomer in which a five-membered ribose is substituted with a six-membered azarsugar, antisense oligomers, the process for preparation thereof. The closest documents related to this invention is document D1 and D2.

Even the document D1 and D2 disclosed antisense drugs, oligomers with modified sugar in the oligonuceltides, the sugar structure of the document D1 and D2 were different from those of this invetion. Document D1 and D2 discloses modified nucleotide monomer which substitutes the natural sugar with the five membered ribose ring. E

Since claims 1-4, 5-7, 8, 9, 10-12, 13-14 in this invention discloses six-membered azarsugar, different from five membered ribose ring in document D1 and D2, those claims are considered to be novel.

3) Inventive Step

Claims 1-4, 5-7, 8, 9, 10-12, 13-14 relate to a nucleotide monomer in which a five-membered ribose is substituted with a six-membered azarsugar, antisense oligomers, the process for preparation thereof. Document D1 and D2 discloses nucleotide base with the five membered ribose ring.

The major difference of this invention and document D1 and D2 is sugar ring structure, which contributes better functional groups for this invention. The six-membered azarsugar are useful for developing antisense drugs due to high binding affinity to mRNA and good membrane permeability and improved resistance to nuclease. The high binding affinity is shown as high Tm value in this invention, which is important technical effects for this invention.

Therefore, claims 1-4, 5-7, 8, 9, 10-12, 13-14 in this invention appear to involve an inventive step.

4) Industrial applicability

The subject matter of claims 1-4, 5-7, 8, 9, 10-12, 13-14 is considered to be industrially applicable.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 January 2001 (11.01.2001)

PCT

(10) International Publication Number
WO 01/02417 A1

(51) International Patent Classification[7]: C07H 21/00

(21) International Application Number: PCT/KR00/00713

(22) International Filing Date: 3 July 2000 (03.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1999/26947 5 July 1999 (05.07.1999) KR

(71) Applicant *(for all designated States except US)*: **DONGBU HANNONG CHEMICAL CO., LTD.** [KR/KR]; 14f, Hankook Joongkongup B/D, #838, Yuksam-dong, Kangnam-ku, Seoul 135-080 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JUNG, Kyeong-Eun** [KR/KR]; #107-1201 Sejong Apt., Junmin-dong, Yusong-ku, Daejeon-si 305-728 (KR). **YANG, Mirim** [KR/KR]; #103-1202 Sejong Apt., Junmin-dong, Yusong-ku, Daejeon-si 305-728 (KR). **LEE, Kwangjun** [KR/KR]; #103-204 Sejong Apt., Junmin-dong, Yusong-ku, Daejeon-si 305-728 (KR). **KIM, Kichul** [KR/KR]; #106-602 Lotte Apt., Nae-dong, Seo-ku, Daejeon-si 302-761 (KR). **LIM, Hong** [KR/KR]; 104-203 Banpo-Apt., Banpo-dong, Seocho-ku, Seoul 134-040 (KR).

(74) Agent: **LEE, Won-Hee**; 8th Fl., Sung-ji Heights II, 642-16 Yoksam-dong, Kangnam-ku, Seoul 135-080 (KR).

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(54) Title: NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF

(57) Abstract: The present invention relates to antisense monomers and oligomers which can inhibit transcription for the production of disease-inducing proteins. The antisense monomers and oligomers of the present invention have greater binding affinity for RNA, a target of general antisense medicine, than that for DNA. In addition, they have nuclease resistance, and can improve permeability of cell membrane. The monomers and oligomers of the present invention can be used for antisense therapy inhibiting the expression of disease-inducing genes, hybridization of gene cloning and reagents for diagnosis. They also provide useful tools for the proteins.

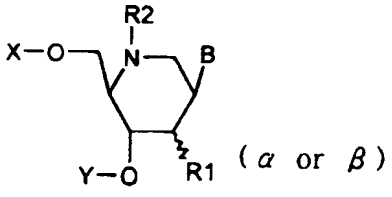
NUCLEOTIDE MONOMER CONTAINING SIX-MEMBERED AZARSUGAR AND ANTISENSE OLIGOMERS THEREOF

FIELD OF THE INVENTION

The present invention relates to a nucleotide monomer represented by the formula 1 in which a five-membered ribose is substituted with a six-membered azarsuger, antisense oligomers represented by the formula 2, and process for preparation thereof.

The antisense oligomers of the present invention are useful for developing antisense drugs since they have high binding affinity to mRNA, good membrane permeability and improved resistance to nuclease.

<FORMULA 1>



wherein,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower

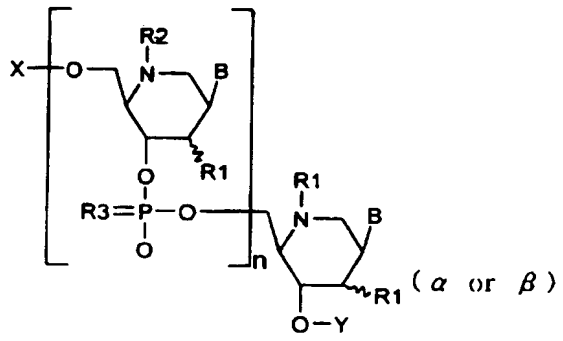
molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) X is hydrogen or hydroxyl protecting group, and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

<FORMULA 2>



wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or

fluorenyl (Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxyl protecting group, conjugate group or oligonuclotide, and

(6) Y is hydrogen, phosphate, activated phosphate, activated phosphite, solid support, conjugate group or oligonucleotide.

BACKGROUND

Proteins comprise more than 20 amino acids and have a very complex and diverse tertiary structure, thus it is very difficult to develop a drug which selectively acts on them. There has been much progress in the development of protein inhibitors as the tertiary structures of various proteins are elucidated by computer simulation and X-ray analysis. However, there has not yet been successful development of effective protein inhibitors.

On the other hand, it has been possible to develop various drugs targeting nucleic acids since nucleic acid comprises 4 different nucleotides of adenosine, guanosine, cytidine and thymidine or uridine, and has a property of complementary binding to each other (Uhlmann *et al.*, "Antisense Oligonucleotides: A New Therapeutic Principles" *Chem. Rev.*, **1990**, 90, 543-584 ; Cohen et al., "The New Genetic Medicine"

Scientific American, **1994**, 271, 76-82).

In vivo protein synthesis accomplishes through expression of the gene which encodes the amino acid sequence. Particulary, one strand of DNA with double-helix structure is transcribed into mRNA and the mRNA is translated to form a protein. On this basis, the drugs aiming at the nucleic acids have been developed and they contain an oligonucleotide with complementary sequence to the mRNA.

The oligonucleotide can bind to the complementary nucleotide of mRNA, inhibit its translation to the protein, and block or reduce the formation of disease-causing proteins. Because the oligonucleotide sequence is reverse (antisense) to the genetic information sequence (sense), the drug is named as antisense drug and the technique antisense technique.

In the late 1970's, Stephenson and Zamecnik found that a synthetic DNA fragment can inhibit the synthesis of viral proteins (Stephenson et al., *Proc. Natl. Acad. Sci. USA,* **1977**, 95, 285 ; Zamecnik et al., *Natl. Acad. Sci. USA,* **1977**, 95, 280). In 1980's, it was also reported that the antisense RNA is synthesized in vivo can regulates the gene expression (Simons et al., *Cell,* **1983**, 34, 683 ; Mizuno et al., *Natl. Acad. Sci. USA,* **1984**, 81, 1966).

However, these natural type oligonucleatides are easily degraded by a nuclease in the body, and a sufficient pharmacological effect can not be expected in spite of their antisense effect. There has been active research effect to produce antisense drugs with improved stability by modifying the structure of antisense oligomers.

The 1st generation antisense drugs are oligomers with phosphate linkage replaced by other groups such as phosphorothioate, methylphosphate, etc. The phosphorthioate is an oligomer whose oxygen of the phosphate group is replaced by sulfur, and it has lower binding affinity to mRNA than the natural type of DNA. However, the phosphrothioate oligomers show strong pharmacological activity *in vivo* or *in vitro*. Some of the 1st generation antisense drugs are being clinically tested as anti-viral or anti-cancer agents and some others are commercially available as anti-viral agents (Bennett et al., "Antisense oligonucleotides: is the glass half full or half empty" *Biochem. Pharmacol.* **1998**, 55, 9-19).

However, these phosphorthioate oligomers also have side effects of toxicity and undesirable immune response (Stein et al., *Current Opinion in Oncology*, **1994**, 6, 587-594 ; Krieg et al, *Nature*, **1995**, 374, 549 ; O'Brien et al., *Leukemia*, **1994**, 8, 2156). New

strategy has been to develop the antisense drugs without these problems and is based on replacing phosphate backbone of the oligonucleotide by amide or ether, modifying the structure of base or ribose (De Mesmaeker et al., "Antisense Oligonucleotides" *Acc. Chem. Res.* **1995**, 28, 366-374).

The 2nd generation antisense drugs are oligomers with modified sugar in the oligonucleotides. They include oligomers containing ribose with methoxy, methoxyethoxy (Martin et al., *Helv. Chim. Acta,* **1995**, 186, 584) or aminoalkoxy (Griffey et al., *J. Med. Chem.* **1996**, 39, 5100-5109) group at 2' position, oligomers containing hexose (Herdewijn et al., In Carbohydrate Modifications in Antisense Research; ACS Symposium Series 580; Sanghvi, Y. S., Cook, P. D., Eds.; American Chemical Society: Washington, DC, 1994; pp 80-99), oligomers containing pentose (Moser et al., Strategies and Chemical Approaches toward Oligonucleotide Therapeutics. In Perspectives in Medicinal Chemistry; Testa, B. et al., Eds.; *Verlag Helvetica Chimica Acta*: Basel, 1993, pp 275-97), oligomers containing 4'-aminoribose (Scharer et al., *J. Am. Chem. Soc.* **1995**, 117, 6623-6624), oligomers containing a 4'-thiobase (Bellon *et al.*, 4-Thio RNA: a novel class of sugar-modified B-RNA. In Carbohydrate Modifications in Antisense Research; ACS Symposium Series 580; Sanghvi, Y. S., Cook, P. D., Eds.; American Chemical Society:

Washington, DC, 1994; pp 68-79) and their derivatives.

mRNA and DNA having the complementary sequence to each other exist in duplex (double strand) form at an ambient temperature (or body temperature). However, they are separated into single strands as the temperature increases, the extent of which is measured following the change in UV absorbance. As the temperature increases, the UV absorbance increases representing sigmoidal curve due to the increase in the amount of single strands whose UV absorbance is higher than that of the duplex. Tm (melting temperature) is defined as the temperature at which 2nd derivative of the the sigmoidal type curve is zero.

High Tm value of the oligomer to mRNA represents high binding afiinity to RNA, and is regarded as a very important factor for the antisense molecules. The binding affinity to mRNA has been measured for the oligomers with various substituents at 2′ position by several researchers (Breslauer et al., *Proc. Natl. Acad. Sci. USA,* **1986**, 83, 3740 ; Freier et al., *Nucleic Acids Res.* **1997**, 25, 4429-4443).

Among the 2nd generation antisense drugs, the oligomers comprising a replaced base with methoxy or fluoro group at 2′ position have high Tm values because electronegative groups introduced at 2′ position increase the binding affinity of the oligomers to RNA

(Kawasaki et al., *J. Med. Chem.*, **1993**, 36, 831-841).

In addition, oligomers with alcoxy group such as methoxy at 2′ position have improved resistance to nuclease when compared with the natural type of DNA. The chimeric oligomer consisted of the modified nucleotides with phosphothioate backbone and the 2′-methoxy substituent shows lower toxicity than that of the 1st generation antisense oligomers (Lesnik et al., *Biochemistry*, **1993**, 32, 7832 ; Milligan et al., *J. Med. Chem.* **1993**, 36, 1923), and its clinical testing was started in 1997 (Agrawal et al., *Curr. Opin. Chem. Biol.*, **1998**, 2, 519).

On the other hand, the oligonucleotide has a potential as diagnostic reagent for genetic deficiencies, primer for PCR, etc. (Englisch et al., *Ang. Chem. Int. Ed.* **1991**, 30, 613-629). It may also be useful for the investigation of the secondary structure of protein and the relationship between protein structure and its activity (Verma et al., "Modified Oligonucleotides", *Ann. Rev. Biochem.*, **1998**, 67, 99-14).

These inventors of the present invention have developed a novel antisense drug with high binding affinity to mRNA, and improved resistance to nuclease resulting in good stability. The oligomer of the present invention contains six-membered azarsugar as a

basic sugar unit substituting the five-membered ribose and shows high binding affinity and good stability.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a nucleotide monomer with six-membered azarsugar replacing five-membered ribose which show high binding affinity to mRNA and improved resistance to nuclease.

It is a further object of this invention to provide antisense oligomer comprising the nucleotide monomer with azarsugar unit partially or as a whole.

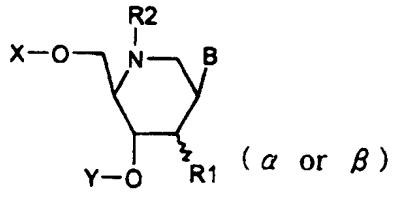
It is an additional object of this invention to provide process for preparing the nucleotide monomer represented by the formula 1 and the antisense oligomer represented by the formula 2.

Further features of the present invention will appear hereinafter.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides a nucleotide monomer with six-membered azarsugar replacing a five-membered ribose represented by the formula 1 and process of preparation thereof.

<FORMULAR 1>



wherein,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl (Fmoc),

(4) X is hydrogen or hydroxyl protecting group,

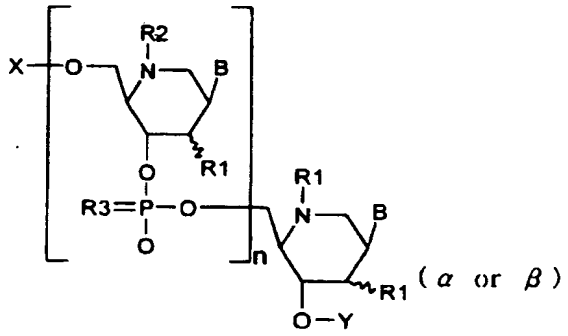
and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

Particulary, R^1 is preferably β-methoxy, or β-ethoxy and R^2 is preferably diphenylmethyl.

In addition, the present invention provides an antisense oligomer represented by the formula 2 which contains the above-mentioned nucleotide monomer partially or as a whole, and process of preparation thereof.

<FORMULA 2>



wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-

ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl (Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxyl protecting group, conjugate group or oligonuclotide, and

(6) Y is hydrogen, phosphate, activated phosphate, activated phosphite, solid support, conjugate group or oligonucleotide.

Particulary, it is preferably R^1 is β-methoxy, β-ethoxy and R^2 is diphenylmethyl.

In the formula 2, n is 1 to 30 including both upper and lower nucleotides, and is preferably 6 to 21. Properties of the oligomer do not depend on the distribution of abovementioned nucleotide monomer in the molecule. However, for the increased binding

affinity, it is desirable to have nucleotides at least 3 bases apart rather than in sequence.

Also, the present invention provides pharmaceutical compositions for effective inhibition of the protein synthesis, which comprises the nucleotide monomer, the antisense oligomer or the chimeric oligomer as an active ingredient.

The present invention also provides the pharmaceutical compositions containing the nucleotide monomer, the antisense oligomer or chimeric oligomer as an active ingredient which is effective for the treatment of hepatitis, cancer or immune diseases by infection of virus or bacteria.

Hereinafter, the present invention is described in detail.

In the present invention, the lower molecular alkyl is defined as an alkly group containing 1-4 carbon atoms and includes methyl, ethyl, propyl, isopropyl, butyl, etc.

The lower molecular alkoxy is an alkoxy group containing 1-4 carbon atoms, includes epoxy, propoxy, butoxy, isopropoxy, etc, and is preferably methoxy or ethoxy.

O-acyl is O-acetyl, O-ethylcarbonyl, O-

propylcarbonyl, etc, aryl is an aromatic hydrocarbon with or without substituents including phenyl, paranitrophenyl and parabromophenyl, araalkyl is alkyl having an aryl group which contains benzyl, ethylphenyl and diphenylmethyl, and is preferably diphenylmethyl.

Heteroaryl is the five-membered or the six-membered ring having one or more of a heteroatom such as sulfur or nitrogen, examples of which are 4-pyridyl and 3-thiophen. Heteroalkyl is alkyl having the five-membered or the six-membered ring having one more of a heteroatom such as sulfur and nitrogen, an example of which includes 4-pyridylmethyl.

The hydroxyl protecting group is one generally known to protect hydroxyl group which includes a 4,4'-dimethoxytrityl group, a lower molecular alkanol, a trimethylsilyl ether (TMS ether), tetra-butyldimethyl silyl ether (TBDMS ether), and is preferably a 4,4'-dimethyltrityl group.

The nucleobase is any natural or modified nucleobases and is preferably a natural nucleobase such as adenine, cytosine, guanine, thymine and uracil or a modified nucleobase with the protecting group, which includes N-benzoiladenine, N-benzoilcytosine and N-isobutyrylguanine. Of the modified nucleobase, 5-(1-propynyl) uracil, 5(1-propynyl)cytosine, inosine, 5-methylicytosine and 2,6-diaminopurine are used commonly.

The oligonucleotide is the natural oligonucleotide

of 1-30 sugar units or its phosphorothioate derivatives.

The solid support may be selected from a controlled pore glass (CPG, in Oligonucleotide synthesis, a practical approach, *M. J. Gait ed.*, Oxford:IRS press, 1984), an oxalyl controlled pore glass(Alul et al., *Nucleic Acids Res.* 1991, 19, 1527), a TentaGel support (Wright et al., *Tetrahedron Lett.* 1993, 34, 3373) composed of aminopolyethyleneglycol derivatives, and a Poros which is a copolymer of polystyrene/divinylbenzene. It is preferably a CPG.

The conjugate group is a group which is bound to the primary or the secondary hydroxyl group via a covalent bond, and promotes absorption of the oligomer. It is includes cholesterol, polylysine, phospholipids, biotin, polyethylene glycol, phenanthroline, phenazine, phenanthridin, anthraquinone, acridine, fluorescein, rhodamine, coumarine and dyes.

The present invention provides a process of preparation for the nucleotide monomer with the six-membered azarsugar. The nucleotide monomer of the present invention can be prepared by a process represented by the reaction schemes 1-5.

The reaction scheme 1 is a process for preparation of the oligomer which comprises the steps of preparing the basic six-membered azarsugar and

binding the adenine nucleobase to a sugar.

The reaction scheme 2 is a process for condensation of the other nucleobase, thymine, cytosine and guanine.

The reaction scheme 3 is a process for binding the various groups to nitrogen of the azarsugar.

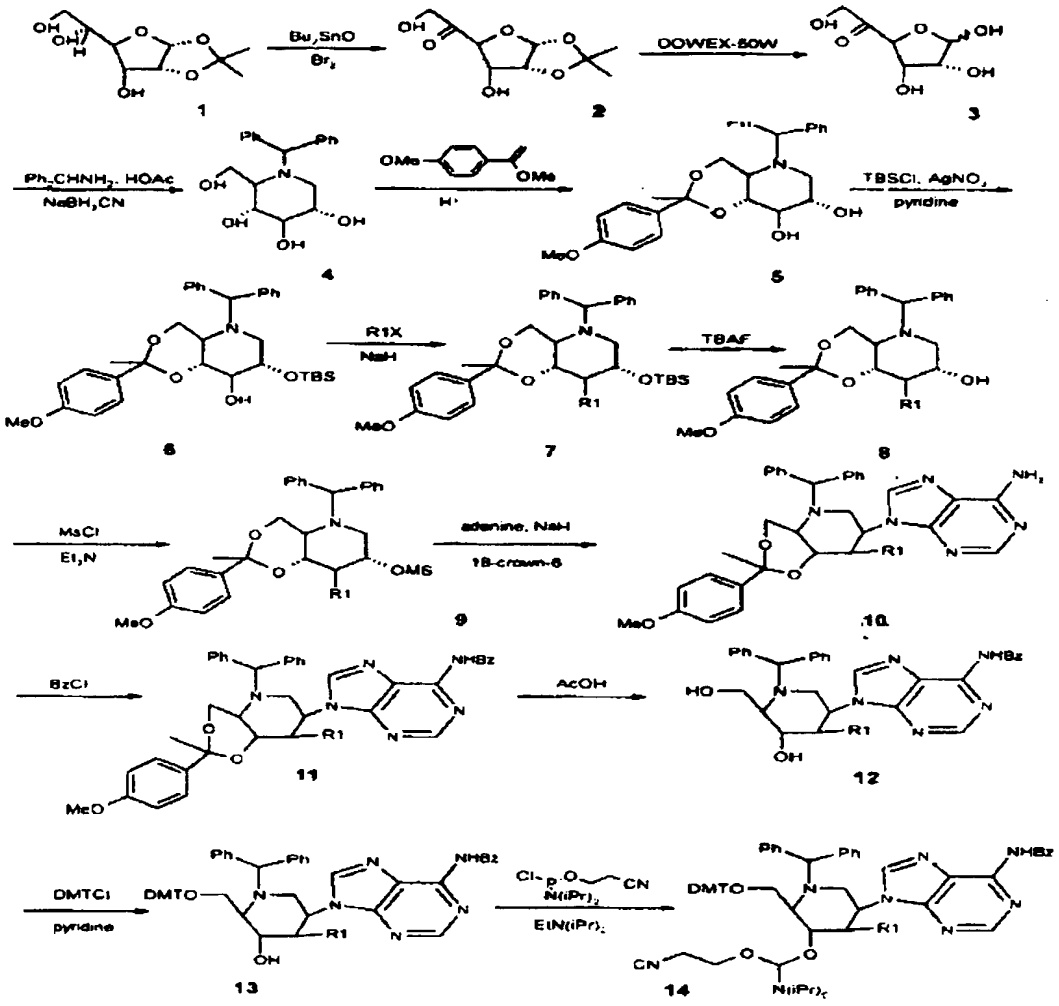
The reaction scheme 4 is a process for removing the hydroxyl group at C-4 of the azarsugar via reduction.

The reaction scheme 5 is a process for changing the synthetic pathway to produce hydroxyl group with opposite stereochemistry.

According to the synthetic pathways represented by reaction scheme 1-5, it is possible to synthesis the monomer represented by the formula 1 which contains all compounds in Examples.

The following explains the reaction scheme 1-5 in more detail.

<REACTION SCHEME 1>



wherein, R^1 is the same as defined in the formula 1.

The reaction scheme 1 is the process for the preparation of the six-membered azarsugar. A deoxynojirimycin derivative (the compound 4 of the reaction scheme 1) with azarsugar as a basic backbone

is synthesized by the known methods using commercially available glucose derivative (Baxter et al., *J. Org. Chem.*, **1994**, 59, 3175-3185).

All intermediates and final products synthesized in the following steps are newly made compounds.

A ketone compound 2 is prepared by oxidation of glucose, compound 1 with a protecting group using dibutyltinoxide (Bu_2SnO) and bromine (Br_2), and the protecting group of the compound 2 is removed with acidic resins to obtain compound 3.

Diphenylmethylamine was added to protect for the compound 3, and ring-shaped six-membered azarsugar 4 was obtained. Compound 5 is obtained by protecting both the primary hydroxyl group at 6' position and the secondary hydroxyl group at 5' position of sugar with α,ρ-dimethoxystyrene.

For the protection of a diol, various other agents beside α,ρ-dimethoxystyrene may be used. However, with other reagents, the reaction does not proceed or the recovery of the product is reduced.

Since the styrene protecting group has a chiral center, the compound 5 is obtained as mixture of 2 diastereomers. However, there is no need to separate the diastereomers because they become one compound in the following deprotection step of styrene protecting group to get compound 12.

Compound 6 protected with

tertbutyldimethyldisilyl (TBS) selectively at the less crowded secondary hydroxyl group of C-3 is synthesized by the reaction of the compound 5 with the tert-butyldimethyl-silyl chloride (TBSCl), $AgNO_3$ and pyridine.

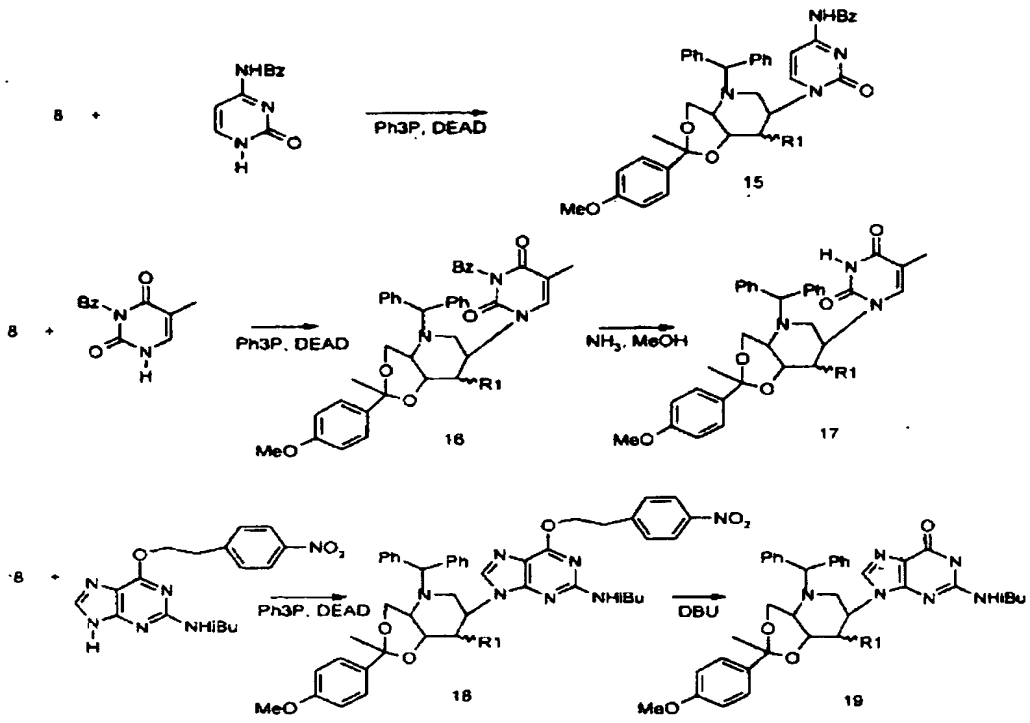
Compound 7 is obtained by alkylating the compound 6 with alkylhalide and NaH, and compound 8 is prepared by removing the TBS protecting group at 3′ position with the reaction of tertbutylammonium fluoride(TBAF).

Compound 9 is obtained by methane sulfonylation of the hydroxyl group of compound 8, and compound 10, a nucleoside, is prepared by condensation with adenine using sodium hydride and 18-crown-6.

Compound 11, a nucleoside, is prepared by monobenzoylation of the amine group of adenine, and compound 12 is prepared by removing the protecting group at 5′ and 6′ hydroxyl groups with 80% acetic acid.

Compound 13 is obtained by treating the nucleoside 12 with 4,4′-dimethoxytrityl chloride, and a phosphoramidite compound 14 is prepared by treating with 2-cyanoetyldiisopropylchlorophosphoramidite ($ClP(OCH_2CH_2CN)N(iPr)_2$) and diisopropylethylamine ($EtN(iPr)_2$).

<REACTION SCHEME 2>



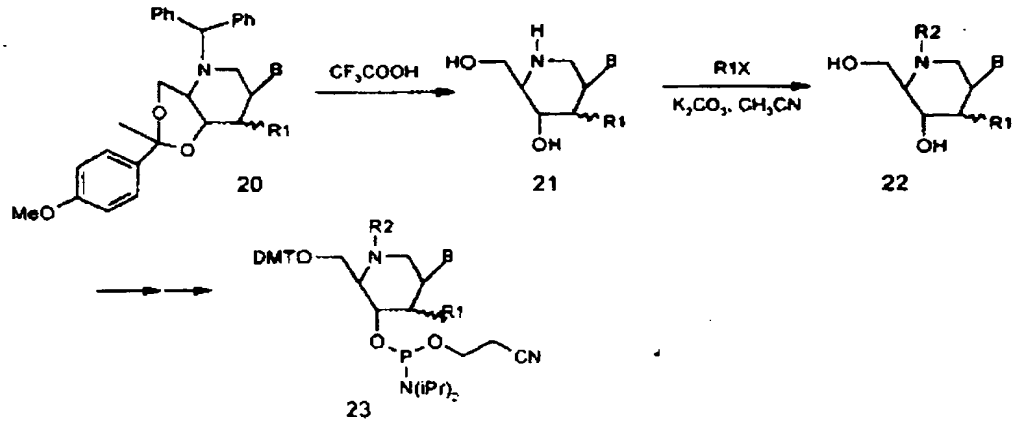
wherein, R^1 is the same as defined the formula 1.

The reaction scheme 2 represents the synthetic pathways for the condensation of azarsugar with other nucleobases (cytosine, thymine, and guanine) except adenine. The process in the scheme 1 may be used, but their reaction yields are low and there is possibility of forming isomers. It is desirable to use Mitsunobu method according to the reaction scheme 2 for the condensation of cytosine, thymine and guanine monomer.

Compound 8 of reaction scheme 1 is allowed to react with N-benzoylcytosine, N-benzoylthymine, and N^2-

isobutyryl-O^6[2-(p-nitrophenyl)ethyl]guanine to form the corresponding condensation products in the presence of triphenylphosphine and DEAD (diethyl azodicarboxylate) in the tetrahydrofuran as solvent. Benzoylthymidine derivative 16 is converted into thymidine derivative 17 by removing the benzoly protecting group with ammonia gas. Compound 19 is synthesized by removing the carbonyl protecting group of guanosine derivative 18 using DBU (1,8-diazabicycle[5.4.0]undec-7-ene). Compounds 15, 17 and 19 are used to form monomers which may be utilized for the synthesis of oligomers either as in the reaction scheme 1 or as in the reaction scheme 3 shown in the following.

<REACTION SCHEME 3>

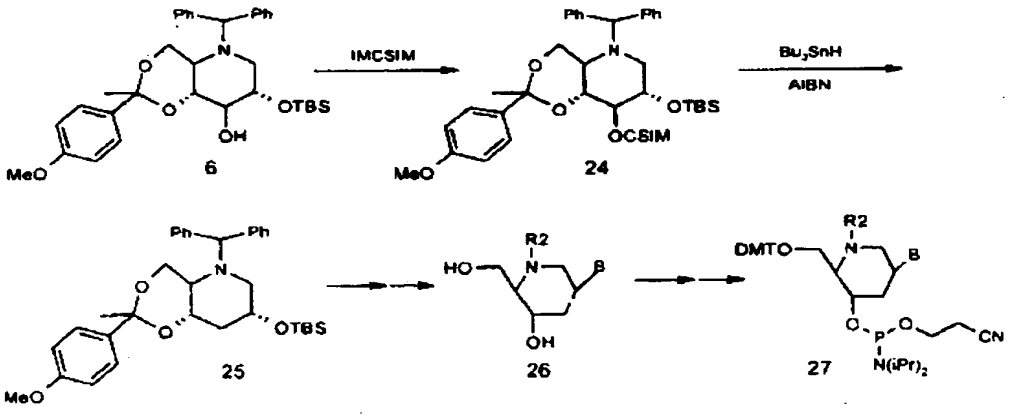


wherein, R^1, R^2 and B is the same as defined the formula 1

The reaction scheme 3 represents the process for introducing various R^2 groups at the nitrogen of six-membered sugar ring of the nucleoside derivatives, which are obtained in the reaction scheme 1 or 2.

Compound 21 is formed when compound 20 is treated with trifluoroacetic acid (CF_3COOH). Compound 22 is synthesized by alkylation of the azarsugar with alkylhalide in the presence of potassium carbonate, or triethylamine or dimethylamino pyridine (DMAP). Compound 23 is synthesized by the same steps as in the reaction scheme 1.

<REACTION SCHEME 4>

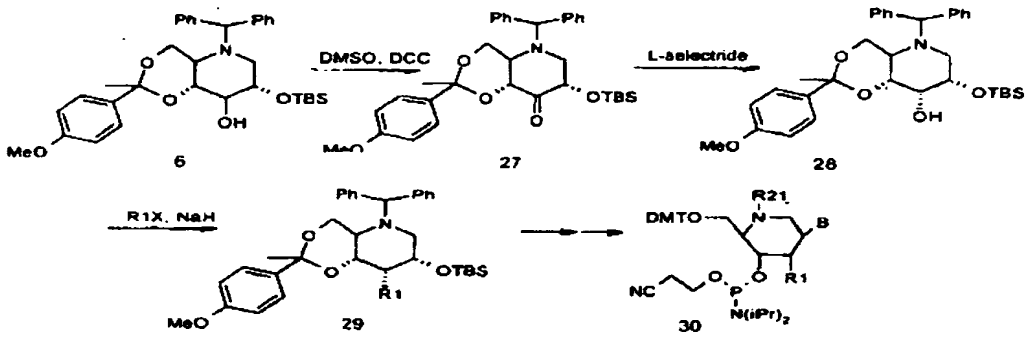


wherein, R^2 and B is the same as defined the

formula 1

The reaction scheme 4 is a process for removing the hydroxyl group at C-4 of azarsugar by reduction. Compound 24 is prepared by the reaction of azarsugar compound 6 with thiocarbondiimidazole, and compound 25 is synthesized by reduction with tributyltin hydride (nBu_3SnH). A compound 26 and 27 are synthesized as in the steps of the reaction scheme 1, 2 or 3.

<REACTION SCHEME 5>



wherein, R^1, R^2 and B is the same as defined the formula 1

The reaction scheme 5 is the process for altering the stereochemistry of the hydroxyl group at position 4 of azarsugar. Compound 27 is obtained from compound 6 according to the oxidation reaction of Swern. Compound

28, having the hydroxyl group with opposite orientation to that of compound 6, is obtained by the reduction reaction of compound 27 with L-selectride. Compound 29 is obtained by alkylation of compound 28, and a phosphoramidite compound 30 is obtained by the reaction cited in the previous schemes.

The present invention provides the process for preparing antisense oligomers, a part or whole of which is composed of nucleotide monomers with six-membered azarsugar instead of ribose, a natural five-membered sugar.

The oligomer of the present invention is prepared by solid phase or liquid phase method, solid phase method being preferable. The details of the oligonucleotide synthesis with the solid phase method are described in "Oligonucleotide Synthesis, A Practical Approach", Gait (ed.), IRL Press, Washington D. C. (1984), Caruthers and et al., U.S. Pat. No.4,458,066 and 4,500,707.

In order to prepare the oligomer via condensation reaction of the nucleotide monomer of the present invention, there is need to protect one primary hydroxyl group of the nucleotide sugar with a dimethoxytrityl group, one secondary hydroxyl group with phosphoramidite group, and nucleobases except thymine with other suitable protecting groups.

To introduce the monomer of the formula 1 at 3'-position of the oligonucleotide, it needs to be secured at a solid support. The monomer is transformed to a hemisuccinate using a controlled pore glass (CPG) with amino group, which can be purchased. Then, the reaction is completed by condensation with mesitylene-2-sulfonyl chloride/1-methyl-1H-imidazole.

To introduce the monomer of the formula 1 at the other positions except the 3'-end of the oligonucleotide, the standard phosphoramidite process is performed using DNA synthesizer (For example, ABI 392). Generally, concentration of the monomer with formula 1 and its reaction time with solid resins are the same as those of common phosphoramidite process of DNA synthesizer. However, if the groups other than hydrogen are present at 2' position, the condensation reaction time of the monomer is needed to be extended to 600 from 60 sec in the original reaction.

Once the desired oligomer is produced, the solid support and the protecting group need to be removed, which may be done simultaneously or in two separation steps. In general, the solid support and the protecting group are removed by the treatment with ammonium hydroxide at ambient temperature (2 hours) and at 55°C (17 hours), respectively.

The protecting group for 5'-hydroxyl group of the oligomer is a dimethoxytrimethyl group, which can be

eliminated in the final step of DNA synthesis by using the program built-in the DNA synthesizer or by treating with 80% acetic acid, dichloroacetic acid or trichloroacetic acid.

If has hydroxyl group at 2'-position, the oligomer is prepared in the DNA synthesizer using isobutyl group for the monomer preparation, which is removed by ammonia in the late stage of the general DNA synthesis process. For the nitrogen of azarsugar of the monomer, fluorenyl group (F-moc) is used for protection and the elimination is carried out by usual method.

In the process of the present invention to prepare the monomer and the antisense oligomer, the present inventors make the process simple by attaching the nucleobases to 3'-carbon position of the six-membered azarsugar (piperidine). If the base is introduced at the aminal position, the reaction product would be α- and β-mixture, requiring separation. Using the process of the invention, the present inventors are able to produce the desired nucleotide with the base only at the carbon position, not at the aminal position. In addition, for the introduction of various group at the nitrogen position, the present inventors make use of azarsugar which can be easily modified without the trouble of using strong basic reagents. The oligomers

with various group introduced at nitrogen position usually have a high Tm to RNA, indicating high affinity to mRNA, and may prove to be effective antisense drugs. The membrane permeability is improved when the present inventors use six-membered azarsugar, substituting ribose, to which hydrophobic group is attached.

The oligomer composed of carbocyclic nucleotides is known to be resistant to nuclease activities. Also, the antisense oligomer of the present invention with phosphate groups is replaced by phosphorothioate partially or as a whole, and has both increased binding affinity and the increased stability to nuclease.

The nucleotide and the antisense oligomer of the present invention can be also prepared in the form of chimeric oligomer which contains phosphodiester or phosphothioate oligonucleotide in the middle of the molecule.

As the cited above, the present invention provides the nucleotide monomer having the six-membered azarsugar replaced by the natural five-membered ribose and the novel oligomer composed of the nucleotide monomer partially or as a whole. The modified nucleotide can bind more strongly to the target RNA than the natural type of DNA, and it also has higher resistance to the degrading enzyme nuclease. It has improved cell membrane permeability when the lipophilic

group is introduced at the nitrogen position of azarsugar.

The oligomer of the present invention can be used for any application requiring antisense oligonucloetide, and it has good characteristics as antisense drugs because of its high binding affinity to mRNA.

When the disease-inducing proteins and the genes involved in their synthesis are discovered, the antisense treatment drugs can be developed by designing to act selectively on the important part (4-30 bases) such as an initiator codon (Akhtar et al, *Nature Genetics*, **1993**, 4, 215). The site of drug action on the mRNA can be determined by several methods (Mishra et al, *C. R. Acad. Sci. III*, 1994, 317, 977 ; Milner et al, *Nat. Biotechnol.*, **1997**, 15, 537). However, it often shows a good antisense effect when the initiation codon or the transcriptional start site is selected as the attacking site (Bacon et al, *Oncogene Res.*, **1991**, 6, 13).

If the sequence of the target mRNA for the antisense oligomer is not known, it can be determined by the analysis of the protein sequence using the genetic code. If the protein sequence is not known, the protein can be used purified and its sequence determined by the known methods.

In addition, the mRNA or DNA responsible for the

protein synthesis may be isolated and identified using the known methods, and the sequences thereof can be used for developing the antisense drugs.

The antisense oligomer compatible with the mRNA sequence is preferably composed of 4-30 units of the monomer, and more preferably 7-22 units.

The oligomer of the present invention can bind to DNA or RNA of various cells including normal cells, cancer cells, tumor cells, protoplasmic cells, amorphous cells and virus. The binding sequences are bacterial sequences, viral sequences, cancer cell sequences and chromosomal sequences. The binding of the oligomer of the present invention to DNA or RNA can inhibit protein synthesis, or can promote the specific protein synthesis by inhibiting the expression of the inhibitor protein.

In addition, the oligomer of the present invention can be used for cure of an infectious disease induced by virus or bacteria, cancer, immune diseases and coronary restenosis. The viral diseases include AIDS, hepatitis B and C, Herpes virus and cytomegalovirus. Cancer contains oncogenes such as c-myc and c-erbB-2 involved the target sequence to DNA or RNA, tumor suppress genes, protein genes-involving genes (protein kinase A, protein kinase C, c-rat kinase, bcl-2, bcr, abl, etc), and the autoimmune diseases contain rheumatoid arthritis, psoriasis, crohn disease,

polyneuritis, the 1st type of diabetes mellitus and lupus.

Moreover, the present invention provides pharmaceutical compositions containing the nucleotide monomer of the formula 1 and the antisense oligomer of the formula 2 as an active ingredient.

The nucleotide monomer, the antisense oligomer or the chimeric oligomer of the present invention can be administered orally or parenterally, and be used in general form of pharmaceutical formulation.

The compounds of the present can be prepared for oral or parenterally administration by mixing with generally-used fillers, extenders, binders, wetting agents, disintegrating agents, diluents such as surfactant, or excipients.

The present invention also includes pharmaceutical formulations in dosage units. This means that the formulations are present in the form of individual parts, for example tablets, coated tablets, capsules, pills, suppositories and ampules, the active compound content of which corresponds to a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses or 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is administered in one application and which

usually corresponds to a whole, one half, one third or a quarter of a daily dose.

Preferred pharmaceutical formulations which may be mentioned are tablets, coated tablets, capsules, pills, granules, suppositories, solutions, suspensions and emulsions, pastes, ointments, gels, creams, lotions, dusting powders and sprays.

Solid formulations for oral administration are tablets, pill, dusting powders and capsules, liquid formulation for oral administrations are suspensions, solutions, emulsions and syrups, and the abovementioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally-used simple diluents such as water and liquid paraffin.

Tablets, coated tablets, capsules, pills and granules can contain the active compound or compounds in addition to the customary excipients, such as (a)fillers and extenders, for example starches, lactose, sucrose, glucose, mannitol and silicic acid, (b)binders, for example carboxymethylcellulose, alginates, gelatine and polyvinylpyrrolidone, (c)humectants, for example glycerol, (d)disintegrating agents, for example agar-agar, calcium carbonate and sodium carbonate, (e)solution retarders, for example paraffin, and (f)absorption accelerators, for example quaternary ammonium compounds, (g)wetting agents, for example

cetyl alcohol and glycerol monostearate, (h) adsorbents, for example kaolin and bentonite, and (i) lubricants, for example talc, calcium stearate, magnesium stearate, and solid polyethylene glycols, or mixtures of the substances listed under (a) to (i).

The tablets, coated tablets, capsules, pills and granules can be provided with the customary coatings and shells, optionally containing opacifying agents, and can also be of a composition such that they release the active compound or compounds only or preferentially in a certain part of the intestinal tract, if appropriate in a delayed manner, examples of embedding compositions which can be used being polymeric substances and waxes.

If appropriate, the active compound or compounds can also be present in microencapsulated form with one or more of the abovementioned excipients.

Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, and suppositories.

Suppositories can contain, in addition to the active compound or compounds, the customary water-soluble or water-insoluble excipients, for example polyethylene glycols, fats, for example cacao fat, and higher esters (for example C14-alcohol with C16-fatty acid) or mixtures of these substances.

Ointments, pastes, creams and gels can contain, in

addition to the active compound or comounds, the customary excipients, for example animal and vegetable fats, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures of these substances.

Dusting powders and sprays can contain, in addition to the active compound or compounds, the customary excipients, for example lactose, talc, silicic acid, aluminum hydroxide, calcium silicate and polyamide powder, or mixtures of these substances. Sprays can additionally contain the customary propellants, for example chlorofluorohydrocarbons.

Solutions and emulsions can contain, in addition to the active compound or compounds, the customary excipients, such as solvents, solubilizing agents and emulsifiers, for example water, ethyl alcohol, isopropyl alcohol, ethylcarbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil and sesame oil, glycerol, glycerol formal, tetrahydrofurfuyl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances.

For parenteral administration, the solutions and emulsions are also be in a sterile form which is

isotonic with blood.

Suspensions can contain, in addition to the active compound or compounds, the customary excipients, such as liquid diluents, for example water, ethyl alcohol and propylene glycol, and suspending agents, for example ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances.

The formulation forms mentioned can also contain coloring agents, preservatives and additives which improve the smell and taste, for example peppermint oil and eucalyptus oil, and sweeteners, for example saccharin.

The abovementioned pharmaceutical formulations can also contain other pharmaceutically active compounds in addition to the compounds according to the present invention.

The abovementioned pharmaceutical formulations are prepared in the customary manner by known methods, for example by mixing the active compound or compounds with the excipient or excipients.

The effective dose of the nucleotide monomer, the antisense oligomer, or the chimeric oligomer of the present invention for use of protein synthesis inhibitors or blockers and for treatment agents of

hepatitic diseases caused by virus or bacteria, cancer or immune diseases is 0.1-50 mg/kg, and is preferably 0.2-2 mg/kg.

The oligomer of the present invention is useful for studying of proteins because it can bind to proteins as well as nucleic acids in cell. That time, the proteins contain receptors, enzymes, ligands and so on.

In addition, because it is stable to PCR and can bind to the specific sequence, the oligomer of the present invention can be used for PCR as the primer.

The oligomer of the present invention also can be used for diagnosis test using the nucleic acid hybridization as a probe (*Nucleic Acids Res.*, **1995**, 23, 217).

Moreover, the oligomer of the present invention can be useful for the various purposes regardless of the absence or the presence of the protecting group, and can be used after the purification step. The purification process is performed by thin layer chromatography, reverse phase high-pressure liquid chromatography (HPLC), ion exchange chromatography or electrophoresis.

EXAMPLES

Practical and presently preferred embodiments of

the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Synthesis of the monomer nucleotide

Example 1 : Preparation of 6-N-bezoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 1, 2-isopropylidene-5-keto-α-D-glucofuranose

Dibutyltinoxide (Bu_2SnO, 48.6 g, 195 mmol) was added to 1, 2-isopropylidene-D-glucufuranose (20 g, 91 mmol) dissolved in methanol (500 mL) and was refluxed for 1 hour. After the reaction mixture was cooled at ambient temperature, the solvent was evaporated under reduced pressure. Methylene chloride (CH_2Cl_2, 500 mL) was added to the residue and this solution was cooled at 0℃. After then, bromine (Br_2, 5.2 mL. 102 mmol) dissolved in methylene chloride (CH_2Cl_2, 100 mL) was slowly added to it. When the addition was completed, the reaction mixture was stirred at the same temperature for 10 min. The solvent was evaporated

under reduced pressure. Methanol and hexane were added little by little to the residue (solid) until two layers of the organic solution was obtained without any solid (upper layer). The hexane layer wituout any solid (upper layer) was removed, the methanol layer (lower layer) was concentrated under reduced pressure, the residue was purified by column chromatography (1%/ 10% MeOH/CH_2Cl_2) to give the desired compound (10.4 g, 53 %). The above method was referred to the reference (Baxter, E. W., Reitz, A. B. J. *Org. Chem.* (1994), vol 59, p. 3175), but it could decrease the separating time and improve the production yield by work-up after the reaction.

^1H NMR (D_2O) δ 1.30 (s, 3H), 1.44 (s, 3H), 4.42 (d, 1H, J=3 Hz), 4.59 (d, 1H, J=3.2 Hz), 4.68 (m, 1H), 4.94 (d, 1H, J=3.3 Hz), 6.06 (d, 1H, J=3.5 Hz).

(Step 2) Preparation of 5-keto-D-glucose

Dowex 50WX 8-200 resin (69.83 g) was added to the title compound of the step 1 (13.65 g, 62.55 mmol) which was dissolved in distilled water (200 mL). The reaction mixture was stirred at ambient temperature for 36 hours. The resin in the reaction mixture was removed by filtration and the residual solution was freeze-dried to give the desired compound (10.3 g, 92 %).

^{1}H NMR (D_2O) δ 3.12 (t, 1H, J=8.8 Hz), 3.40 (brt, 2H, J=9.9 Hz), 3.51 (d, 1H, J=11.8 Hz), 3.57 (t, 1H, J=10.1 Hz), 4.83 (d, 1H, J=8.2 Hz)

(Step 3) Preparation of (3S,4R,5R,6R)-2-(hydroxymethyl)-N-benzhydrylpiperidine-3,4,5-triol

The title compound (8.24 g, 46.26 mmol) prepared from the step 2 was dissolved, and the reaction mixture was added to a solution of aminodiphenylmethane (6.76 g, 36.89 mmol) and acetic acid (2.22 g, 36.97 mmol) in methanol (300 mL). Sodium hydride ($NaCNBH^3$, 5.82 g, 92.62 mmol) was added to the reaction mixture, stirred at -78℃ for 2 hours, and warmed to ambient temperature. The reaction mixture was stirred at ambient temperature for 2 days, concentrated under reduced pressure. Saturated sodium bicarbonate (Na_2CO_3) solution was added. The solution was extracted with methylene chloride. The organic layer was dried by sodium sulfate (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2, 100% - 10% MeOH/CH_2Cl_2) to give the desired compound (5.9 g, 39%).

^{1}H NMR (D_2O) δ 1.85 (brt, 1H, J=10.5 Hz), 2.38 (brd, 1H, J=9.2 Hz), 2.93 (dd, 1H, J=4.3, 11.3 Hz), 3.06 (brt, 1H, J=8.1 Hz), 3.51 (brm, 2H), 3.67 (brt, 1H,

J=5.5 Hz), 3.83 (brd, 1H, J=4.5 Hz), 3.95 (brd, 1H, J=10.1 Hz), 3.99 (brd, 1H, J=4.8 Hz), 4.11 (brs, 1H), 4.22 (brd, 1H, J=11.5 Hz), 5.71 (s, 1H), 7.10 - 7.24 (m, 10H).

(Step 4) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3,4-dihydroxyl-5,6-O-[(4-methoxylphenyl)ethylidene]piperidi-ne

The title compound of the step 3 (3.5 g, 1.63 mmol) was dissolved in fresjly distilled methlyene chloride (100 mL) and α,p-dimethoxystyrene (1.86 mL) and pyridinium p-toluenesulfonate (0.92 g, 3.67 mmol) were added to this reaction mixture. The reaction mixture was stirred at ambient temperature for 16 hours, and was extracted with the saturated sodium carbonate solution and methylene chloride. The organic layer solvent was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (10% MeOH/CH_2Cl_2) to give the desired compound (diastereomer A:B = 1.7:1, 3.7g, 76%).

^{1}H NMR ($CDCl_3$) diastereomer A δ 1.79 (s, 3H), 1.92 (ddd, 1H, J=4.8, 10.7, 10.7 Hz), 2.46 (m, 1H), 2.58 (m, 1H), 3.07 (dd, 1H, J=4.8, 11.3 Hz), 3.37 (dd, 1H, J=8.8, 9 Hz), 3.62 (dd, 1H, J=10.2, 10.5 Hz), 3.81 (s, 3H, OMe), 3.90 (dd, 1H, J=9.2, 9.2 Hz), 4.50 (dd, 1H, J=4.7, 10.8 Hz), 5.04 (s, 1H), 6.89 (d, 2H, J=8.6

Hz), 7.15 - 7.38 (m, 10H), 7.50 (d, 2H, J=8.6 Hz); diastereomer B δ 1.54 (s, 3H), 1.92 (ddd, 1H, J=4.8, 10.7, 10.7 Hz), 2.46 (m, 1H), 2.89 (m, 1H), 2.99 (dd, 1H, J=4.8, 11.3 Hz), 3.37 (dd, 1H, J=8.8, 9 Hz), 3.51 (dd, 1H, J=9, 9 Hz), 3.85 (s, 3H, OMe), 3.99 (dd, 1H, J=10.5, 10.5 Hz), 4.44 (dd, 1H, J=3.9, 10.2 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.5 Hz), 7.15 - 7.38 (m, 12H).

(Step 5) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-hydroxyl-5,6-O-[(4-methoxylphenyl)ethylidene]piperidine

Pyridine (8.81 g, 9.01 mmol) and silver nitrate ($AgNO_3$, 5.68 g, 33.42 mmol) were added to title compound of the step 4, a diasteromer (13.18 g, 28.55 mmol)(or mixture of diastereomer) in tetrahydrofuran (300 mL). The reaction mixture was stirred for 20 min at ambient temperature and tert-butyldimethylsilylchloride (TBDMSiCl, 5.68 g, 37.73 mmol) was added. After stirring for 12 hrs at ambient temperature, reaction solution was filtered and the filtrate was concentrated under reduced pressure. Methylene chloride and sodium bicarbonate solution were added and shaked. The organic layer was separated, dried and concentrated under reduced pressure. The reaction mixture was purified by column chromatography (10 % ethylacetate/hexane) to give the desired compound (12 g, 73 %).

^{1}H NMR ($CDCl_3$) diastereomer A δ 0.00 (s, 3H, Si-Me), 0.07 (s, 3H, Si-Me), 0.84 (s, 9H, Si-tBu), 1.78 (s, 3H, Me), 1.92 (dd, 1H, J=11, 11 Hz), 2.45 (ddd, 1H, J=4.4, 9.7, 9.7 Hz), 2.61 (brs, 1H, OH), 2.90 (dd, 1H, J=4.7, 9.7 Hz), 3.39 (dd, 1H, J=8.7, 8.7 Hz), 3.77 (m, 1H), 3.81 (s, 3H, OMe), 3.98 (dd, 1H, J=10.2, 10.6 Hz), 4.44 (dd, 1H, J=4.5, 10.5 Hz), 5.03 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 (d, 2H, J=8 Hz), 7.30 - 7.47 (m, 8H), 7.49 (s, 2H, J=8.8 Hz); diastereomer B δ -0.07 (s, 3H, Si-Me), 0.03 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.54 (s, 3H, Me), 1.90 (dd, 1H, J=10.5, 10.5 Hz), 2.48 (ddd, 1H, J=4, 10.4, 10.4 Hz), 2.82 (dd, 1H, J=4.8, 11.4 Hz), 3.38 (dd, 1H, J=9, 9 Hz), 3.52 (dd, 1H, J=8.9, 9.4 Hz), 3.55 (m, 1H), 3.62 (dd, 1H, J=10.5, 10.5 Hz), 3.86 (s, 3H, OMe), 4.39 (dd, 1H, J=4, 9.6 Hz), 4.94 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.15 - 7.47 (m, 12H).

(Step 6) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyoxy-4-methoxy-5,6-O-[(4-methoxyphe-nyl)ethylidene]piperidene

Iodomethane (2.74 g, 19.3 mmol, 1.2 mL) and sodium hydride (0.933 g, 233.25 mmol) were added to the title compound of the step 5, diastereomer A (5.6 g, 9.66 mmol), in anhydrous tetrahydrofuran. The reaction mixture was stirred at ambient temperature for 2 hours under nitrogen. After adding brine to the reaction

mixture, it was extracted with methylene chloride, washed the organic layer with water, dried and concentrated under reduced pressure. The residue was purified by column chromatography (10 % ethylacetate/hexane) to give the desired compound(5.38 g, 94 %).

^{1}H NMR ($CDCl_3$) diastereomer A δ 0.00 (s, 3H, Si-Me), 0.06 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.75 (s, 3H, Me), 1.91 (dd, 1H, J=10.8, 10.8 Hz), 2.42 (ddd, 1H, J=3.6, 9.3, 9.3 Hz), 2.87 (dd, 1H, J=5, 11.4 Hz), 3.00 (dd, 1H, J=8.7, 8.7 Hz), 3.63 (s, 3H, OMe), 3.57 (m, 1H), 3.81 (s, 3H, OMe), 3.93 (dd, 1H, J=9, 9 Hz), 3.96 (dd, 1H, J=10, 10 Hz), 4.45 (dd, 1H, J=4.6, 10.8 Hz), 5.02 (s, 1H), 6.89 (d, 2H, J=8.9 Hz), 7.14 - 7.47 (m, 10H), 7.48 (d, 2H, J=8.8 Hz); diastereomer B δ -0.07 (s, 3H, Si-Me), 0.05 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.50 (s, 3H, Me), 1.83 (dd, 1H, J=11, 11 Hz), 2.43 (m, 1H, J=3.6, 9.3, 9.3 Hz), 2.79 (dd, 1H, J=5, 11.3 Hz), 2.97 (dd, 1H, J=8.7, 8.7 Hz), 3.52 (dd, 1H, J=9, 9 Hz), 3.53 (dd, 1H, J=10, 10 Hz), 3.67 (s, 3H, OMe), 3.77 (m, 1H), 3.86 (s, 3H, OMe), 4.38 (dd, 1H, J=4, 10.5 Hz), 4.91 (s, 1H), 6.98 (d, 2H, J=8.9 Hz), 7.14 - 7.46 (m, 12H).

(Step 7) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]

piperidene

Tetrabutylammonium fluoride (1 M solution dissolved in tetrahydrofuran, 25 mL, 25 mmol) was added to the title compound of step 6 in tetrahydrofuran. After stirring at ambient temperature for 2.5 hrs, the reaction mixture was evaporated under reduced pressure. The residue was purified by column chromatography (10 - 40 % ethylacetate/hexane) to give the desired compound (3.44 g, 86 %).

^{1}H NMR ($CDCl_3$) diastereomer A δ 1.77 (s, 3H, Me), 1.93 (dd, 1H, J=10.8, 10.8 Hz), 2.47 (ddd, 1H, J=4.7, 10.1, 10.1 Hz), 3.02 (dd, 1H, J=9, 9 Hz), 3.08 (dd, 1H, J=5, 11.2 Hz), 3.71 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.00 (dd, 1H, J=10.5, 10.5 Hz), 4.03 (dd, 1H, J=9, 9 Hz), 4.51 (dd, 1H, J=4.5, 10.8 Hz), 5.05 (s, 1H), 6.89 (d, 2H, J=8.8 Hz), 7.14 - 7.47 (m, 10H), 7.47 (d, 2H, J=8.8 Hz); diastereomer B δ 1.58 (s, 3H, Me), 1.91 (dd, 1H, J=10.5, 10.5 Hz), 2.48 (ddd, 1H, J=4, 10.3, 10.3 Hz), 3.02 - 2.98 (m, 2H), 3.70 - 3.57 (m, 3H), 3.82 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.45 (dd, 1H, J=4, 10.5 Hz), 4.95 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.16 - 7.39 (m, 12H).

(Step 8) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethyl-idene]piperidine

Methanesulfonyl chloride (2.15 g, 18.81 mmol) and triethylamine (1.46 mL) were added to the title compound of the step 7 (2.98 g, 6.27 mmol) which was dissolved in distilled methylene chloride (100 mL), and was stirred for 1 hours. The reaction mixture was extracted with sodium bicarbonate solution and methylene chloride. The organic layer was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (20 % - 50 % ethlyacetate/hexane) to give the desired compound (3.44 g, 99 %).

^{1}H NMR ($CDCl_3$) diastereomer A δ 1.77 (s, 3H, Me), 2.07 (dd, 1H, J=11, 11 Hz), 2.44 (ddd, 1H, J=4.7, 9.9, 9.9 Hz), 3.09 (s, 3H, OMs), 3.21 (m, 1H), 3.22 (dd, 1H, J=9, 9 Hz), 3.67 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.99 (dd, 1H, J=10.6, 10.6 Hz), 4.03 (dd, 1H, J=9.1, 9.1 Hz), 4.52 (dd, 1H, J=4.5, 10.9 Hz), 4.59 (ddd, 1H, J=5.2, 10.5, 10.5 Hz), 5.07 (s, 1H), 6.89 (d, 2H, J=8.9 Hz), 7.15 - 7.38 (m, 10H), 7.46 (d, 2H, J=8.9 Hz); diastereomer B δ 1.53 (s, 3H, Me), 2.04 (dd, 1H, J=10.9, 10.9 Hz), 2.45 (ddd, 1H, J=4, 10.2, 10.2 Hz), 3.07 (s, 3H, OMs), 3.19 (dd, 1H, J=9.2, 9.2 Hz), 3.60 (dd, 1H, J=4, 10.4 Hz), 3.62 (dd, 1H, J=9.1, 9.1 Hz), 3.73 (s, 3H, OMe), 3.87 (s, 3H, OMe), 4.40 (dd, 1H, J=5.2, 9.3 Hz), 4. 43 (ddd, 1H, J=4.2, 10.8, 10.8 Hz), 4.96 (s, 1H), 6.99 (d, 2H, J=8.8 Hz), 7.14 - 7.39 (m,

12H).

(Step 9) Preparation of {(3R,4R,5R,6R)-N-banzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

Adenine (0.82 g, 6.07 mmol), sodium hydride (304 mg, 7.6 mmol) and 18-crown-6 (319 mg, 1.21 mmol) were dissolved in anhydrous N,N-dimethylformamide (75 mL) and stirred at 80℃ for 1 hour. The title compound of the step 8, diastereomer B (1.67 g, 3.02 mmol)(diastereomer A or A and B mixture) dissolved in anhydrous N,N-dimethlyformamide (25 mL) was added to the reaction mixture, heated at 100℃ and stirred for 16 hours. After cooling and concentrating the reaction mixture under reduced pressure, it was dissolved in ethylacetate (400 mL) and washed with the saturated sodium bicarbonate solution (25 mL) and water (50 mL). After evaporating the organic solvent under reduced pressure, the residue was purified by column chromatography (5 % methanol/methylene chloride) to dive the desired compound (900 mg, 50 %).

^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H), 2.74 (m, 2H), 2.80 (dd, 1H, J=11.3, 11.5 Hz), 2.98 (m, 1H), 3.37 (s, 3H, OMe), 3.69 (dd, 1H, J=10.4, 10.4 Hz), 3.92 (m, 2H), 3.93 (s, 3H, OMe), 4.47, (ddd, 1H, J=4, 10.6, 10.6 Hz), 4.48 (m, 1H), 5.02 (s, 1H), 6.99 (d, 2H, J=8.8 Hz)),

7.13 - 7.42 (m, 12H), 7.71 (s, 1H), 8.32 (s, 1H).

(Step 10) Preparation of 6-N-benzoly-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidinr-3-yl}adenine

The title compound of the step 9 (1.65 g, 2.78 mmol) was dissolved in pyridine (100 mL) and cooled at 0℃. After droping benzoyl chloride (1.17 g, 8.34 mmol) to the reaction mixture for 30 min, it was stirred for 2 hours. The reaction mixture was cooled at 0℃, added with water (2.7 mL) and stirred for 5 min. After adding ammonia water(5.56 mL) to the reaction mixture at ambient temperature, it was stirred for 15 min, added with water (500 mL) and extracted with methylene chloride (200 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (10 % methanol/methlenechloride) to give the desired compound (1.27 g, 66 %).

^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H), 2.75 (ddd, 1H, J=3, 9.1, 9.1 Hz), 2.82 (dd, 1H, J=11.4, 11.4 Hz), 3.05 (dd, J=4.5, 11.3 Hz), 3.39 (s, 3H, OMe), 3.71 (dd, 1H, J=10.5, 10.5 Hz), 3.82 (dd, 1H, J=9, 9 Hz), 3.88 (s, 3H, OMe), 3.96 (dd, 1H, J=9.1, 10 Hz), 4.49 (m, 2H), 5.03 (s, 1H), 7.00 (d, 2H, J=8.7 Hz), 7.13 - 7.41 (m, 12H), 7.52 (t, 2H, J=6.7 Hz), 7.60 (d, 1H, J=7.1 Hz), 7.91 (s,

1H), 8.02 (d, 2H, J=7.3 Hz), 8.75 (s, 1H).

(Step 11) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidi-ne-3-yl}adenine

The title compound of the step 10 (1.22 g, 1.75 mmol) was dissolved in 80 % acetic acid (10 g) and stirred at 45 - 50℃ for 3 hours. After adding methylene chloride (100 ml) and the saturated sodium bicarbonate solution (20 mL) to the reaction mixture. The organic layer was separated, dried and concentrated under reduced pressure. The residue was purified by column chromatography (5 % methanol/methylene chloride) to give the desired compound (543 mg, 55 %).

^{1}H NMR ($CDCl_3$) δ 2.74 (m, 1H), 2.97 (dd, 1H, J=11, 11 Hz), 3.10 (s, 3H, OMe), 3.19 (dd, 1H, J=4, 12 Hz) 3.78 (dd, 1H, J=8.5, 9.7 Hz), 4.02 (dd, 1H, J=8.5, 8.5 Hz), 4.17 (dd, 1H, J=2, 12 Hz), 4.27 (dd, 1H, J=3.5, 12 Hz), 4.61 (ddd, 1H, J=4.2, 10.5, 10.5 Hz), 5.55 (s, 1H), 7.19 - 7.53 (m, 10H), 7.55 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7 Hz), 8.02 (s, 1H), 8.04 (d, 2H, J=7.2 Hz), 8.76 (s, 1H).

(Step 12) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-metho-xypiperidine-3-yl}adenine

4,4'-Dimethoxytrityl chloride (0.752 g, 2.22 mmol) was added to the title compound of the step 11 (500 mg, 0.89 mmol) which was dissolved in pyridine (10 mL) at 0℃. The reaction mixture was stirred for 16 hours, and concentrated under reduced pressure. 5 % Sodium bicarbonate (10 mL) solution was added to the reaction mixture. The reaction mixture was extracted with methylene chloride (100 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane:TEA = 50:50:1) to give the desired compound (672 mg, 88 %).

^{1}H NMR ($CDCl_3$) δ 2.68 (m, 1H), 2.78 (m, 1H), 2.97 (dd, 1H, J=11, 11 Hz), 3.09 (s, 3H, OMe), 3.12 (dd, 1H, J=4.2, 11.8 Hz) 3.65 (dd, 1H, J=3.2, 10.4 Hz), 3.793 (s, 3H, OMe), 3.796 (s, 3H, OMe), 4.21 (dd, 1H, J=8.5, 8.5 Hz), 4.63 (ddd, 1H, J=4.2, 10.3, 10.3 Hz), 5.09 (s, 1H), 6.81 (d, 2H, J=8.7 Hz), 6.82 (d, 2H, J=9 Hz), 7.18 - 7.32 (m, 16H), 7.37 (d, 2H, J=8.9 Hz), 7.38 (d, 2H, J=8.9 Hz), 7.51 (t, 2H, J=6.8 Hz), 7.59 (d, 1H, J=7.2 Hz), 8.01 (s, 1H), 8.02 (d, 2H, J=7 Hz), 8.78 (s, 1H).

(Step 13) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

After evaporating the title compound of the step 12 (654 mg, 0.754 mmol) with anhydrous toluene three times using vacuum pump, it was dissolved in methylene chloride(7 mL) under nitrogen, and N,N-diisopropylethlyamine (356 μl) was added to the reaction micture. 2-Cyanoethyl N,N-diisopropyl chloro phosphoramidite (356 μl) was added to the reaction mixture, and it was stirred for 5 hours under nitrogen. The reaction solution was washed with 5 % sodium bicarbonate solution (10 mL) and extracted with methylene chloride (100 mL). The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane:TEA = 50:50:1) to give the desired compound (diastereomer mixture 465 mg, 58 %).

^{1}H NMR ($CDCl_3$) δ 1.04 (d, 6H, J=6.7 Hz), 1.15 (d, 6H, J=6.5 Hz), 1.19 (d, 6H, J=6.5 Hz), 1.21 (d, 6H, J=6.7 Hz), 2.52 (t, 4H, J=6 Hz), 2.63 (d, 1H, J= 11.1 Hz), 2.73 (dd, 1H, J=2, 13 Hz), 2.84 (dd, 1H, J=4, 6 Hz), 2.92 (dd, 1H, J=3.5, 13.5 Hz), 3.27 (s, 3H, OMe), 3.33 (s, 3H, OMe), 3.36 - 3.80 (m, 11H), 3.834 (s, 6H, OMe), 3.838 (s, 6H, OMe), 3.84 (m, 2H), 4.34 (d, 2H, J=11.1 Hz), 4.40 (d, 2H, J=10.6 Hz), 4.68 (s, 1H), 4.70 (s, 1H), 4.77 (m, 2H), 6.83 (d, 8H, J=8.8 Hz), 7.12 - 7.39 (m, 30H), 7.55 (t 4H, J=7 Hz), 7.62 (d, 2H, J=7.2 Hz), 8.07 (d, 4H, J=7.3 Hz), 8.72 (s, 1H), 8.75 (s, 1H),

9.06 (s, 1H), 9.32 (s, 1H).

^{31}P NMR ($CDCl_3$) δ 149.01, 150.07.

Example 2 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the diastereomer A (diastereomer B or A and B mixture) of the Example 1 (step 5) as a starting material via the procedure described in the Example 1 using iodoethane (EtI).

^1H NMR ($CDCl_3$) δ -0.02 (s, 3H, Si-Me), 0.07 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.24 (t, 3H, J=7.1 Hz, $OCH_2\underline{C}H_3$), 1.75 (s, 3H, Me), 1.91 (dd, 1H, J=10.8, 10.8 Hz), 2.43 (ddd, 1H, J=4.6, 9.4, 9.4 Hz), 2.88 (dd, 1H, J=4.8, 11.5 Hz), 3.08 (dd, 1H, J=8.7, 8.7 Hz), 3.74 (m, 1H), 3.82 (s, 3H, OMe), 3.94 (m, 4H), 4.45 (dd, 1H, J=4.2, 10.3 Hz), 5.03 (s, 1H), 6.88 (d, 2H, J=8.9 Hz), 7.14 - 7.40 (m, 10H), 7.46 (d, 2H, J=8.9 Hz).

(Step 2) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]

piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.76 (s, 3H, Me), 1.93 (dd, 1H, J=10.8, 10.8 Hz), 2.45 (ddd, 1H, J=4.4, 9.7, 10.1 Hz), 3.11 (m, 2H), 3.71 (m, 1H), 3.82 (s, 3H, OMe), 3.99 (m, 1H), 4.03 (dd, 1H, J=4.2, 9 Hz), 4.12 (q, 2H, J=7 Hz), 4.51 (dd, 1H, J=4, 6 Hz), 5.05 (s, 1H), 6.90 (d, 2H, J=9 Hz), 7.14 - 7.36 (m, 10H), 7.45 (d, 2H, J=9 Hz).

(Step 3) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-ethoxy-5,6-O-[(4methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.77 (s, 3H, Me), 2.07 (dd, 1H, J=11.1, 11.1 Hz), 2.43 (ddd, 1H, J=4.5, 9.7, 9.7 Hz), 3.11 (s, 3H, OMs), 3.25 (dd, 1H, J=5.3, 11.3 Hz), 3.32 (dd, 1H, J=9, 9 Hz), 3.71 (q, 2H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 4.03 (m, 2H), 4.03 (dd, 1H, J=9, 9 Hz), 4.52 (dd, 1H, J=4.4, 11 Hz), 4.58 (ddd, 1H, J=5.2, 9.8, 9.8 Hz), 5.07 (s,

1H), 6.89 (d, 2H, J=8.8 Hz), 7.13 - 7.37 (m, 10H), 7.44 (d, 2H, J=8.8 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.80 (s, 3H, Me), 2.71 (ddd, 1H, J=4.4, 9.8, 9.8 Hz), 2.90 (dd, 1H, J=11.3, 11.3 Hz), 3.10 (dd, 1H, J=4.7, 11.1 Hz), 3.23 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.76 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 3.95 (dd, 1H, J=9.9, 9.9 Hz), 4.07 (dd, 1H, J=10.6, 10.6 Hz), 4.20 (dd, 1H, J=8.9, 8.9 Hz), 4.53 (dd, 1H, J=4.5, 11 Hz), 4.64 (ddd, 1H, J=4.5, 10.8, 10.8 Hz), 5.13 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 - 7.47 (m, 12H), 7.73 (s, 1H), 8.31 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.79 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 1.81 (s, 3H, Me), 2.73 (ddd, 1H, J=4.4, 9.7, 9.7 Hz), 2.93 (dd, 1H, J=11.5, 11.5 Hz), 3.15 (dd, 1H, J=4.4, 11.3 Hz), 3.22 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.80 (q, 1H, J=7 Hz, $OCH_2C\underline{H}_3$), 3.82 (s, 3H, OMe), 3.98 (dd, 1H, J=9.1, 9.1 Hz), 4.08 (dd, 1H, J=10.6, 10.6 Hz), 4.23 (dd, 1H, J=8.9, 8.9 Hz), 4.54 (dd, 1H, J=4.5, 10.9 Hz), 4.72 (ddd, 1H, J=4.6, 8.8, 9.9 Hz), 5.14 (s, 1H), 6.88 (d, 2H, J=8.8 Hz), 7.14 - 7.56 (m, 15H), 7.95 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.76 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 11 via the procedure described in the step 5 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 2.73 (m, 1H), 2.98 (m, 2H), 3.05 (d, 1H, J=11.2 Hz), 3.20 (dd, 1H, J=4.1, 11.6 Hz), 3.37 (dq, 1H, J=2.3, 7 Hz, $OCH_2C\underline{H}_3$), 3.81 (dd, 1H, J=9.5, 9.5 Hz), 4.01 (dd, 1H, J=8.4, 8.4 Hz), 4.28 (dd, 1H, J=3.3, 12.1 Hz), 4.59 (ddd, 1H, J=4, 10.3, 10.3 Hz), 5.56 (s, 1H), 7.20 - 7.64 (m, 13H), 8.01 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.76 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-ethoxy piperidine-3-yl}denine

This compound was prepared from the title compound of the step 12 via the procedure described in the step 6 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, J=7 Hz, $OCH_2C\underline{H}_3$), 2.51 (d, 1H, J=3 Hz), 2.64 (d, 1H, J=8.5 Hz), 2.91 (dq, 1H, J=2.3, 7 Hz), 3.06 (dd, 1H, J=10.9 Hz), 3.15 (dd, 1H, J=4.6, 11.4 Hz), 3.41 (dq, 1H, J=2.3, 7 Hz, $OCH_2C\underline{H}_3$), 3.66 (dd, 1H, J=3.2, 10.4 Hz), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.81 (m, 1H), 4.22 (dd, 1H, J=2.5, 7.4 Hz), 4.62 (ddd, 1H, J=4.4, 10.4, 10.4 Hz), 5.09 (s, 1H), 6.82 (d, 2H, J=9 Hz), 6.83 (d, 2H, J=9 Hz), 7.18 - 7.65 (m, 22H), 8.01 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.79 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperi -dine-3-yl}adenine

This compound was prepared from the title compound of the step 13 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.01 (t, 3H, J=6.8 Hz, $OCH_2C\underline{H}_3$), 1.03 (d, 6H, J=6.7 Hz), 1.13 (d, 6H, J=6.7 Hz), 1.16 (d, 6H, J=6.7 Hz), 1.20 (d, 6H, J=6.7 Hz), 2.52 (m, 4H), 2.60 (d, 2H, J=11.1 Hz), 2.78 (dd, 2H, 3.7, 11.5 Hz), 2.94 (dd, 2H, J=3.6, 11.4 Hz), 3.01 (dd, 2H, J=4.7, 11.1 Hz), 3.18 - 3.80 (m, 16H), 3.83 (s, 6H, OMe), 3.84 (s, 6H, OMe), 4.28 (d, 2H, J=13.6 Hz), 4.41 (d, 2H, J=13.5 Hz), 4.72 (m, 2H), 4.76 (s, 2H), 6.82 (d, 8H, J=8.7 Hz), 7.08 - 7.66 (m, 44H), 8.07 (d, 4H, J=7.2 Hz), 8.73 (s, 1H), 8.75 (s, 1H), 9.08 (s, 1H), 9.34 (s, 1H).

^{31}P NMR ($CDCl_3$) δ 148.85, 149.96.

Example 3 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxy piperidine-3-yl}adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-banzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxymethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

The diastereomer B (diastereomer A or A and B mixture)(200 mg, 0.61 mmol) obtained from the step 5 of the Example 1 was dissolved in anhydrous tetrahydrofuran (5 mL), and sodium hydride (72 mg, 1.8 mmol) in tetrahydrofuran (2 mL) was added to the reaction mixture. After heating the reaction mixture at 60℃, 2-bromoethylmethylether (171 μL, 1.8 mmol)

was added to the reaction solution, and it was stirred at 60℃ for 1 day. After adding water, the reaction mixture was extracted with ethylacetate, dried by sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 5 - 10% ethylacetate/hexane solvent to give the desired compound (215 mg, 56 %).

^{1}H NMR ($CDCl_3$) δ 0.13 (s, 3H, Si-Me), 0.20 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.51 (s, 3H, Me), 1.83 (dd, 1H, J=11, 11 Hz), 2.41 (m, 1H), 2.87 (dd, 1H, J=5, 11.4 Hz), 3.03 (dd, 1H, J=4.7, 11.5 Hz), 3.23 (s, 3H, OMe), 3.26 - 3.61 (m, 4H), 3.73 (ddd, 1H, J=5, 8.7, 8.7 Hz), 3.85 (s, 3H, OMe), 3.92 (d, 1H, J=9 Hz), 3.98 (d, 1H, J=10.7 Hz), 4.42 (m, 1H), 4.95 (s, 1H), 6.93 (d, 2H, J=8.8 Hz), 7.16 - 7.40 (m, 12H).

(Step 2) Preparation of (3S,4R,5R,6R)-N-benahydryl-3-hydroxyl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.51 (s, 3H, Me), 1.88 (dd, 1H, J=10.8, 10.8 Hz), 2.45 (ddd, 1H, J=2.8, 9.2, 9.2 Hz), 2.76 (m, 1H), 3.02 (dd, 1H, J=5, 11.8 Hz), 3.11 (dd, 1H,

J=9, 9 Hz), 3.43 (s, 3H, OMe), 3.55 - 3.68 (m, 4H), 3.86 (s, 3H, OMe), 3.90 (m, 1H), 4.26 (ddd, 1H, J=3, 5.4, 11.8 Hz), 4.44 (dd, 1H, J=4, 11 Hz), 4.93 (s, 1H), 6.97 (d, 2H, J=8.7 Hz), 7.20 - 7.40 (m, 12H).

(Step 3) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-methoxyethoxy-5,6-O-[(4methoxyphenyl) ethlyidene]piperihine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H, Me), 2.04 (dd, 1H, J=11, 11 Hz), 2.46 (ddd, 1H, J=4, 10, 10 Hz), 3.15 (s, 3H, OMs), 3.33 (d, 1H, J=11 Hz), 3.41 (s, 3H, OMe), 3.61 - 3.68 (m, 2H), 3.86 (s, 3H, OMe), 3.88 (m, 1H), 4.18 (dd, 1H, J=3, 5.7 Hz), 4.22 (dd, 1H, J=3, 5.7 Hz), 4.39 (dd, 1H, J=4.5, 9.5 Hz), 4.45 (dd, 1H, J=4, 10 Hz), 4.95 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.14 - 7.40 (m, 12H).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-banzhydryl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.54 (s, 3H, Me), 2.75 (ddd, 1H, J=4, 10, 10 Hz), 2.95 (m, 1H), 3.12 (s, 3H, OMe), 3.14 - 3.22 (m, 2H), 3.48 (ddd, 1H, J=3, 5.8, 5.8), 3.81 (dd, 1H, J=6.2, 6.2 Hz),3.85 (m, 1H), 3.87 (s, 3H, OMe), 3.94 (ddd, 1H, J=3.2, 6, 11.4 Hz), 4.13 (dd, 1H, J=9.5, 9.5 Hz), 4.42 (dd, 1H, J=5, 10.2 Hz), 4.47 (dd, 1H, J=4, 10.5 Hz), 5.00 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.14 - 7.44 (m, 12H), 7.74 (s, 1H), 8.30 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxyethoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H, Me), 2.74 (ddd, 1H, J=5, 9, 9 Hz), 2.97 (dd, 1H, J=11.2, 11.2 Hz), 3.03 (m, 1H), 3.11 (s, 3H, OMe), 3.14 - 3.21 (m, 2H), 3.46 (ddd, 1H, J=3.1, 6, 11.5), 3.70 (dd, 1H, J=10.5, 10.5 Hz), 3.83 (d, 1H, J=8.9 Hz), 3.88 (s, 3H, OMe), 3.98 (ddd, 1H, J=3, 5.5, 5.5 Hz), 4.17 (dd, 1H, J=9.2, 9.2 Hz), 4.51 (m, 1H), 4.54 (ddd, 1H, J=5, 11, 11 Hz), 5.02 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.14 - 7.62 (m, 12H), 7.96 (s, 1H), 8.03 (d, 2H, J=7.4 Hz), 8.76 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxyethoxypi-peridine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.69 (m, 1H), 2.90 (dd, 1H, J=11.5, 11.5), 3.17 (dd, 1H, J=4, 11.5 Hz), 3.31 (s, 3H, OMe), 3.24 - 3.40 (m, 4H), 3.89 (dd, 1H, J=9, 9 Hz), 4.01 (dd, 1H, J=8.7, 8.7 Hz), 4.23 (m, 2H), 4.57 (ddd, 1H, J=4, 10.7, 10.7 Hz), 5.59 (s, 1H), 7.17 - 7.38 (m, 10H), 7.51 (t, 2H, J=7.2 Hz), 7.59 (d, 1H, J=7.3 Hz), 7.90 (s, 1H), 8.05 (d, 2H, J=7.2 Hz), 8.72 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.60 (m, 1H), 3.02 (m, 1H), 3.16 (dd, 1H, J=4.3, 11.3), 3.36 (s, 3H, OMe), 3.26 - 3.38 (m, 4H), 3.57 (d, 1H, J=9.5 Hz), 3.79 (s, 3H, OMe), 3.86 (d, 1H, J=9.8 Hz), 3.95 (dd, 1H, J=10, 10 Hz), 4.33 (dd, 1H, J=8.8, 8.8 Hz), 4.63 (ddd, 1H, J=4.2,

10.8, 10.8 Hz), 5.02 (s, 1H), 6.79 (d, 2H, J=8.8 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.22 - 7.42 (m, 12H), 7.53 (t, 2H, J=7.3 Hz), 7.61 (d, 1H, J=7.3 Hz), 7.97 (s, 1H), 8.03 (d, 2H, J=7.3 Hz), 8.78 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.01 (d, 6H, J=6.8 Hz), 1.15 (d, 6H, J=6.8 Hz), 1.19 (d, 6H, J=6.8 Hz), 1.24 (d, 6H, J=6.8 Hz), 2.49 (m, 4H), 2.60 (m, 2H), 2.77 (m, 3H), 2.94 (m, 2H), 3.21 (s, 3H, OMe), 3.23 (s, 3H, OMe), 3.27 - 3.59 (m, 18H), 3.83 (s, 12H, OMe), 4.17 (m, 1H), 4.28 (d, 2H, J=13.4 Hz), 4.40 (d, 2H, J=12.3 Hz), 4.75 (s, 2H), 4.77 (m, 2H), 6.81 (d, 8H, J=8.4 Hz), 7.06 - 7.35 (m, 38H), 7.53 (d, 4H, J=7.5 Hz), 7.61 (d, 2H, J=7.5 Hz), 8.05 (d, 4H, J=7.3 Hz), 8.75 (s, 1H), 9.02 (s, 1H), 9.13 (s, 1H), 9.29 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 148.99, 149.77.

Example 4 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,Ndiisopropylamino)

phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl} adenine

(Step 1) Preparation of (3S,4R,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-(imidazole-1-yl)-thiocarboxyl-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

1,1'-Thiocarbonyldiimidazole (12.44 g, 69.81 mmol) was added to the title compound, diastereomer A (12.0 g, 20.84 mmol)(diastereomer B or A and B mixture) obtained from the step 6 of the example 1 which was dissolved in acetonitrile (200 mL). The reaction mixture was refluxed for 24 hours and concentrated under reduced pressure. Methylene chloride and water were added to the reaction mixture, and the organic layer was extracted. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hexane = 1:4) to give the desired compound (3.33 g, 23 %).

^{1}H NMR ($CDCl_3$) δ -0.18 (s, 6H, Si-Me), 0.68 (s, 9H, Si-tBu), 1.45 (s, 3H, Me), 2.12 (dd, 1H, J=11, 11 Hz), 2.65 (ddd, 1H, J=4, 10.1, 10.1 Hz), 2.94 (dd, 1H, J=5, 11.6 Hz), 3.70 (dd, 1H, J=10.6, 10.6 Hz), 3.74 (m, 1H), 3.43 (s, 1H, OMe), 3.44 (m, 1H), 4.99 (s, 1H), 5,78 (dd, 1H, J=9, 9 Hz), 6.93 (d, 2H, J=8.8 Hz), 7.16 - 7.43 (m, 12H), 7.76 (s, 1H), 8.46 (s, 1H).

(Step 2) Preparation of (3S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-5,6-O-[(4-methoxyphenyl)ethyl-idene]piperidine

Tributyltin hydride (2.22g 7.62 mmol) and 2,2'-azobis(isobutyronitryl)(0.82 g, 4.97 mmol) was added to the title compound of the step 1 (2.62g 3.82 mmol) which was dissolved in toluene (30 mL) and stirred for 2 hours. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (10% - 20% EtOAc/Hexane) to give the desired compound (2.0 g, 47 %).

^{1}H NMR ($CDCl_3$) δ -0.03 (s, 3H, Si-Me), 0.00 (s, 3H, Si-Me), 0.82 (s, 9H, Si-tBu), 1.55 (s, 3H, Me), 1.81 (dd, 1H, J=10.5, 10.5 Hz), 2.18 (m, 1H), 2.33 (ddd, 1H, J=4, 10.5, 10.5 Hz), 2.86 (dd, 1H, J=4, 10.3 Hz), 3.49 (ddd, 1H, J=4.4, 10.5, 10.5 Hz), 3.61 (dd, 1H, J=10.5, 10.5 Hz), 3.69 (m, 1H), 3.86 (s, 3H, OMe), 4.50 (dd, 1H, J=4, 10.5 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.8 Hz), 7.16 - 7.47 (m, 12H).

(Step 3) Preparation of (3S,5R,6R)-N-benzhydryl-3-hydroxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H, Me), 1.76 (dd, 1H, J=10.5, 10.5 Hz), 2.32 (m, 2H), 3.00 (dd, 1H, J=3, 10.5 Hz), 3.51 - 3.59 (m, 2H), 3.66 (m, 1H), 3.76 (m, 1H), 3.86 (s, 3H, OMe), 4.47 (dd, 1H, J=4, 10.5 Hz), 4.97 (s, 1H), 6.96 (d, 2H, J=6.8 Hz), 7.17 - 7.40 (m, 12H).

(Step 4) Preparation of (3S,5R,6R)-N-benzhydryl-3-methanesulfoxy-5,6-O-[(4-methoxyphenyl)ethylidene]pipe-ridine

This compound was prepared via the procedure described in the step 8 of the Example.

^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H, Me), 2.01 (dd, 1H, J=10.5, 10.5 Hz), 2.38 (ddd, 1H, J=4, 10.2, 10.2 Hz), 2.53 (m 1H), 2.95 (s, 3H, OMs), 3.14 (dd, 1H, J=4, 11.5 Hz), 3.56 (m, 1H), 3.63 (dd, 1H, J=10.5, 10.5 Hz), 3.87 (s, 3H, OMe), 4.46 (dd, 1H, J=4, 10.5 Hz), 4.67 (m, 1H), 4.98 (s, 1H), 6.96 (d, 2H, J=8.6 Hz), 7.19 - 7.42 (m, 12H).

(Step 5) Preparation of {(3R,5R,6R)-N-benzhydryl-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.54 (s, 3H, Me), 2.24 (dd, 1H, J=10.8, 10.8 Hz), 2.55 (m, 2H), 3.20 (m, 1H), 3.72 (dd, 1H, J=10.5, 10.5 Hz), 3.86 (m 1H), 3.88 (s, 3H, OMe), 4.53 (dd, 1H, J=4, 10.5 Hz), 4.77 (m, 1H), 5.06 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.12 - 7.39 (m, 12H), 8.03 (s, 1H), 8.32 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,5R,6R)-N-benzhydryl-5,6-O-[(4-methoxyohenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H, Me), 2.27 (dd, 1H, J=11, 11 Hz), 2.55 (m, 2H), 3.21 (m, 1H), 3.74 (dd, 1H, J=10.5, 10.5 Hz), 3.78 (m, 1H), 3.88 (s, 3H, OMe), 4.54 (dd, 1H, J=4, 10.5 Hz), 4.77 (m, 1H), 5.07 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.14 - 7.55 (m, 15H), 7.94 (s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.75 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethylpiperidine-3-yl} adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.07 (m, 1H), 2.54 (m, 2H), 2.68 (m, 1H), 3.25 (dd, 1H, J=3, 11.3 Hz), 4.17 - 4.24 (m, 3H), 4.85 (m, 1H), 5.43 (s, 1H), 7.18 - 7.33 (m, 10H), 7.52 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.05 (d, 2H, J=7.2 Hz), 8.26 (s, 1H), 8.70 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethylpiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.45 (m, 2H), 2.63 (dd, 1H, J=7, 12.4 Hz), 2.85 (m, 1H), 3.14 (dd, 1H, J=3, 12, 12 Hz), 3.52 (dd, 1H, J=6, 10 Hz), 3.74 (dd, 1H, J=3, 10 Hz), 3.823 (s, 3H, OMe), 3.829 (s, 3H, OMe), 4.25 (m, 1H), 4.84 (m, 1H), 4.92 (s, 1H), 6.85 (d, 2H, J=8.9 Hz), 6.87 (d, 2H, J=8.9 Hz), 7.15 - 7.35 (m, 12H), 7.44 (d, 2H, J=7 Hz), 7.55 (t, 2H, J=7.3 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.05 (d, 2H, J=7 Hz), 8.52 (s, 1H), 8.78 (s, 1H).

(Step 9) Preparation of 6-N-benzoyl-{(3R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 8 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.05 (d, 6H, J=6.7 Hz), 1.10 (d, 6H, J=6.7 Hz), 1.16 (d, 6H, J=6.7 Hz), 1.19 (d, 6H, J=6.7 Hz), 2.28 (m, 2H), 2.73 (m, 2H), 2.77 (m, 4H), 2.96 (dd, 2H, J=2, 12.8 Hz), 3.10 (dd, 2H, J=3, 13 Hz), 3.33 (dd, 2H, J=8.2, 8.7 Hz), 3.42 - 3.75 (m, 12H), 3.82 (s, 3H, OMe), 3.83 (s, 6H, OMe), 3.84 (s, 3H, OMe), 4.37 (m, 1H), 4.57 (s, 1H), 4.60 (s, 1H), 4.91 (m, 2H), 6.85 (d, 4H, J=7.2 Hz), 6.86 (d, 4H, J=7.2 Hz), 7.07 - 7.42 (m, 34H), 7.55 (d, 4H, J=7.7 Hz), 7.62 (d, 2H, J=7.2 Hz), 8.09 (d, 4H, J=7.3 Hz), 8.71 (s, 1H), 8.72 (s, 1H), 9.17 (s, 1H), 9.30 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 148.30, 148.59

Example 5 : Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

(Step 1) Preparation of (3S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-oxo-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

After adding dimethylsulfoxide (253 ㎕, 3.81 mmol) and oxalyl chloride (1.8 mL, mmol) to anhydrous

dichloromethane (40 mL) at -78 ℃, the title compound, diastereomer B (1 g, 1.73 mmol)(diastereomer A or A and B mixture) obtained from the step 5 in the Example 1 was added to it for 5 min. After stirring for 15 min, riethylamine (574 ㎕, 0.87 mmol) was added to it at -78 ℃. A cooling device was removed after 5 min, and the temperature of reaction mixture was allowed to reach ambient temperature. Distilled water was added to the reaction mixture, the organic solvent layer was separated, dried by sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography (5~10 %) eluted with methanol/dichloromethane solvent system to give the desired compound (700 mg, 71 %).

^{1}H NMR ($CDCl_3$) δ -0.15 (s, 3H, Si-Me), 0.08 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.59 (s, 3H, Me), 2.30 (ddd, 1H, J=11, 11 Hz), 2.75 (ddd, 1H, J=3.6, 10, 10 Hz), 3.21 (dd, 1H, J=7, 11.3 Hz), 3.80 (m, 1H), 3.89 (s, 3H, OMe), 4.16 (dd, 1H, J=1.5, 9.4 Hz), 4.26 (dd, 1H, J=5.2, 10.7 Hz), 4.46 (dd, 1H, 4, 10.6 Hz), 5.10 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17 - 7.41 (m, 12H).

(Step 2) Preparation of (3S,4S,5R,6R)-N-banzhydryl-3-tertbutyldimethylsilyloxy-4-hydroxy-5,6-O-[(4-methoxy phenyl)ethylidene]piperidine

L-Selectride (23.2 mL, 23.2 mmol) was added to

the title compound of the step 1 (3.3 g, 5.8 mmol) which was dissolved in tetrahydrofuran under nitrogen and stirred for 17 hours. Distilled water and methylene chloride were added to the reaction mixture, the organic layer was separated, dried by sodium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 5~10 % ethylacetate/hexane solvent system to give the desired compound (2.2 g, 66 %).

^{1}H NMR ($CDCl_3$) δ -0.36 (s, 3H), 0.03 (s, 3H), 0.84 (s, 9H), 1.58 (s, 3H), 2.29 (dd, 1H, J=10.5, 10.5 Hz), 2.54 (dd, 1H, J=4.8, 11 Hz), 2.89 (ddd, 1H, J=4.2, 10.5, 10.5 Hz), 3.55 (dd, 1H, J=2.5, 9 Hz), 3.61 (dd, 1H, J=10.5, 10.5 Hz), 3.74 (ddd, 1H, J=2.9, 4.7, 10.4 Hz), 3.87 (s, 1H), 4.45 (dd, 1H, J=4.3, 10.4 Hz), 4.95 (s, 1H), 6.97 (d, 2H, J=8.7 Hz), 7,17~7.39 (m, 12H).

(Step 3) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 6 of the Example 1.

^{1}H NMR ($CDCl_3$) δ -0.03 (s, 3H, Si-Me), 0.03 (s,

3H, Si-Me), 0.85 (s, 9H, Si-tBu), 1.58 (s, 3H, Me), 2.33 (dd, 1H, J=11, 11 Hz), 2.51 (dd, 1H, J=4.4, 11 Hz), 2.78 (ddd, 1H, J=4.2, 10.4, 10.4 Hz), 3.47 (dd, 1H, J=2.3 9.4 Hz), 3.57 (m, 2H), 3.59 (s, 3H, OMe), 3.69 (ddd, 1H, J=2.6, 4.5, 4.5 Hz), 3.87 (s, 3H, OMe), 4.41 (dd, 1H, J=4.2 10.5 Hz), 4.93 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17 - 7.38 (m, 12H).

(Step 4) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-hydroxy-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.53 (s, 3H, Me), 2.03 (dd, 1H, J=10.5, 10.5 Hz), 2.74 (ddd, 1H, J=5.4, 11, 11 Hz), 2.78 (m, 1H), 3.55 - 3.68 (m, 4H), 3.64 (s, 3H, OMe), 3.87 (s, 3H, OMe), 4.44 (dd, 1H, J=4.3, 10.4 Hz), 4.93 (s, 1H), 6.95 (d, 2H, J=8.8 Hz), 7.17 - 7.39 (m, 12H).

(Step 5) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-methanesulfoxy-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.57 (s, 3H, Me), 2.52 (dd, 1H, J=10.8, 10.8 Hz), 2.75 (dd, 1H, J=5, 10.7 Hz), 2.84 (ddd, 1H, J=6, 9.6, 9.6 Hz), 2.97 (s, 3H, OMs), 3.57 (d, 1H, J=10.2 Hz), 3.56 (m, 1H), 3.57 (s, 3H, OMe), 3.88 (s, 3H, OMe), 3.94 (m, 1H), 4.44 (dd, 1H, J=4.3, 10.4 Hz), 4.66 (ddd, 1H, J=2.8, 4.6, 10.9 Hz), 4.97 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.16 - 7.40 (m, 12H).

(Step 6) Preparation of {(3R,4S,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.56 (s, 3H, Me), 2.72 (m, 2H), 3.03 (ddd, 1H, J=4.2, 10, 10 Hz), 3.42 (m, 1H), 3.45 (s, 3H, OMe), 3.69 (dd, 1H, J=10.6, 10.6 Hz), 3.76 (m, 1H), 3.86 (s, 3H, OMe), 4.52 (dd, 1H, J=4, 10.4 Hz), 4.90 (m, 1H), 5.04 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.15 - 7.43 (m, 12H), 7.94 (s, 1H), 8.37 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4methoxyphenyl)ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title

compound of the step 6 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.56 (s, 3H, Me), 2.76 (m, 2H), 3.07 (ddd, 1H, J=4.2, 10, 10 Hz), 3.48 (s, 3H, OMe), 3.69 (dd, 1H, J=10.6, 10.6 Hz), 3.79 (m, 2H), 3.90 (s, 3H, OMe), 4.52 (m, 1H), 4.98 (m, 1H), 5.05 (s, 1H), 7.00 (d, 2H, J=8.6 Hz), 7.18 - 7.56 (m, 15H), 8.04 (d, 2H, J=8.5 Hz), 8.17 (s, 1H), 8.81 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.93 (d, 1H, J=9.6 Hz), 2.99 (dd, 1H, J=3.9, 10.9 Hz), 3.20 (s, 3H, OMe), 3.88 (dd, 1H, 3.4, 3.4 Hz), 4.16 (dd, 1H, J=10.7, 10.7 Hz), 4.26 (dd, 1H, J=3.8, 12.2 Hz), 5.08 (dd, 1H, J=4, 9.5 Hz), 5.48 (s, 1H), 7.18 - 7.40 (m, 10H), 7.53 (t, 2H, J=7 Hz), 7.61 (d, 1H, J=7.1 Hz), 8.06 (d, 2H, J=7.3 Hz), 8.03 (s, 1H), 8.80 (s, 1H)).

(Step 9) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-

methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 8 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.89 (m, 1H), 3.01 (m, 1H), 3.18 (m, 2H), 3,23 (s, 3H, OMe), 3.58 - 3.66 (m 2H), 3.82 (s, 6H, OMe), 4.36 (m 1H), 4.85 (m, 1H), 5.14 (m, 1H), 6.85 (d, 2H, J=8.9 Hz), 6.86 (d, 2H, J=8.8 Hz), 7.11 - 7.47 (m, 20H), 7.54 (t, 2H, J=7.5 Hz), 7.61 (d, 1H, J=7.3 Hz), 8.07 (d, 3H, J=7.9 Hz), 8.80 (s, 1H).

(Step 10) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 9 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.99 (d, 6H, J=6.6 Hz), 1.22 (d, 6H, J=6.6 Hz), 1.28 (d, 6H, J=6.8 Hz), 1.29 (d, 6H, J=6.8 Hz), 2.57 (m, 2H), 2.77 (dd, 2H, J=6.4, 6.8 Hz), 2.84 (m, 1H), 3.00 (m, 1H), 3.36 (s, 3H, OMe), 3.44 - 3.66 (m, 4H), 3.75 (m, 1H), 3.83 (s, 6H, OMe), 3.84 (s, 6H, OM e), 4.13 - 4.17 (m, 2H), 4.45 (m, 1H), 4.55 (d, 1H, J=10 Hz), 5.16 (m, 1H), 6.87 (d, 4H, J=7.4 Hz),

7.13 - 7.43 (m, 21H), 7.55 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7 Hz), 8.10 (d, 2H, J=7.3 Hz), 8.73 (s, 1H), 9.18 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 153.08

Example 6 : Preparation of 6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

(Step 1) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-tertbutyldimethylsilyloxy-4-ethoxy-5,6-O-[(4-methoxyphe-nyl)ethylidene]piperidine

This compound was prepared from the title compound of the step 2 in Example 5 via the procedure described in the step 6 of the Example 1 using iodoethane (EtI).

^{1}H NMR ($CDCl_3$) δ -0.05 (s, 3H, Si-Me), 0.01 (s, 3H, Si-Me), 0.83 (s, 9H, Si-tBu), 1.18 (t, 3H, J=7 Hz, OCH_2CH_3), 1.51 (s, 3H, Me), 2.34 (dd, 1H, J=11.2, 11.2 Hz), 2.50 (dd, 1H, J=5, 10 Hz), 2.84 (ddd, 1H, J=4, 10, 10 Hz), 3.46 (dd, 1H, J=9.3 Hz), 3.56 (dd, 1H, J=10.4, 10.4 Hz), 3.67 (m 2H), 3.81 (q, 2H, J=7 Hz), 3.87 (s, 3H, OMe), 4.40 (dd, 1H, J=4, 10.4 Hz), 4.91 (s, 1H), 6.98 (d, 2H, J=8.7 Hz), 7.17 - 7.38 (m, 12H).

(Step 2) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-hydroxy-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.18 (t, 3H, J=7.2 Hz, OCH_2CH_3), 1.52 (s, 3H, Me), 2.04 (dd, 1H, J=11, 11 Hz), 2.72 (dd, 1H, J=5, 11 Hz), 2.80 (m, 1H), 3.53~3.77 (m, 3H), 3.77 (dd, 1H, J=3, 3 Hz), 3.87 (s, 3H), 4.10 (m, 2H), 4.43 (dd, 1H, J=4, 10 Hz), 4.92 (s, 1H), 6.96 (d, 2H, J=8.8 Hz), 7.17~7.39 (m, 12H)

(Step 3) Preparation of (3S,4S,5R,6R)-N-benzhydryl-3-methanesulfonyl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethyl -idene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, J=7 Hz, OCH_2CH_3), 1.62 (s, 3H, Me), 2.56 (dd, 1H, J=11, 11 Hz), 2.76 (dd, 1H, J=5, 10 Hz), 2.91 (m, 1H), 2.95 (s, 3H, OMs), 3.58 (m, 1H), 3.59 (dd, 1H, J=11, 11 Hz), 3.74 (dq, 1H, J=3, 7Hz, OCH_2CH_3), 3.87 (s, 3H, OMe), 3.93 (dq, 1H, J=3, 7 Hz, OCH_2CH_3), 4.03 (m, 1H), 4.39 (m, 1H), 4.65 (m, 1H),

4.96 (s, 1H), 6.97 (d, 2H, J=12.7 Hz), 7.18~7.41 (m, 12H).

(Step 4) Preparation of (3R,4S,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-iladenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, J=7 Hz, OCH_2CH_3), 1.53 (s, 3H, Me), 2.73 (m, 1H), 2.77 (d, 1H, J=12.4 Hz), 3.06 (ddd, 1H, J=4, 9, 9 Hz), 3.33 (dq, 1H, J=7, 11.8 Hz, OCH_2CH_3), 3.68 (dd, 1H, J=11, 11 Hz), 3.84~3.93 (m, 3H), 3.86 (s, 3H, OMe), 4.49 (dd, 1H, J=4.2, 10.5 Hz), 4.89 (ddd, 1H, J=2.4, 5, 11.3 Hz), 5.03 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.15~7.43 (m, 12H), 7.96 (s, 1H), 8.38 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-4-ethoxy-5,6-O-[(4-methoxyphenyl)ethlyidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H, J=7 Hz, OCH_2CH_3),

1.55 (s, 3H, Me), 2.77 (m, 2H), 3.04 (m, 1H), 3.39 (dd, 1H, J=7, 10 Hz), 3.70 (dd, 1H, J=10.5, 10.5 Hz), 3.87 (m, 2H), 3.90 (s, 3H, OMe), 3.96 (dd, 1H, J=7.2, 9.5 Hz), 4.50 (dd, 1H, J=4.2, 10.5 Hz), 4.99 (m, 1H), 5.05 (s, 1H), 6.99 (d, 2H, J=8.7 Hz), 7.17~7.56 (m, 15H), 8.03 (d, 2H, J=7 Hz), 8.19 (s, 1H), 8.82 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J=7 Hz, OCH_2CH_3), 2.91 - 3.18 (m, 3H), 3.19 (dq, 1H, J=7, 7 Hz, OCH_2CH_3), 3.38 (dq, 1H, J=7, 9.3 Hz, OCH_2CH_3), 3.98 (m, 1H), 4.14 (d, 1H, J=11.7 Hz), 4.16 (m, 1H), 4.25 (dd, 1H, J=3.7, 11.9 Hz), 5.08 (m, 1H), 5.48 (s, 1H), 7.8 - 7.63 (m, 13H), 8.06 (d, 2H, J=7 Hz), 8.40 (s, 1H), 8.80 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J=7 Hz, OCH_2CH_3), 2.91 (dd, 1H, J=7, 11.7 Hz), 3.04 (m, 1H), 3.15 (m, 1H), 3.25 (m, 1H), 3.38 (m, 1H), 3.56 (m, 1H), 3.62 (m, 1H), 3.82 (s, 6H, OMe), 3.95 (dd, 1H, J=4, 4 Hz), 4.33 (m, 1H), 4.83 (s, 1H), 5.12 (m, 1H), 6.84 (d, 2H, J=9 Hz), 6.87 (d, 2H, J=9 Hz), 7.08 - 7.58 (m, 18H), 7.47 (d, 2H, J=7 Hz), 7.55 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7.2 Hz), 8.06 (d, 2H, J=7.4 Hz), 8.81 (s, 1H), 9.06 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J=7 Hz, OCH_2CH_3) 0.96 (d, 3H, J=7 Hz, $CH(C\underline{H}_3)_2$), 1.20 (d, 3H, J=7 Hz, $CH(C\underline{H}_3)_2$), 1.22~1.30 (m, 6H, OMe), 1.97 (m, 1H), 2.29 (m, 1H), 2.53 (t, 1H), 2.84 (m, 1H), 2.90 (m, 1H), 3.02 (m, 1H), 3.50~3.59 (m, 7H), 8.84 (s, 6H), 4.08 (m, 1H), 4.38 (m, 1H), 4.51 (m, 1H), 5.13 (m, 1H), 6.87 (d, 4H, J=7 Hz), 7.03~7.40 (m, 19H), 7.56 (t, 2H, J=7.6 Hz), 7.62 (d, 1H, J=7.3 Hz), 8.10 (d, 2H, J=7 Hz), 8.81 (s, 1H), 9.14 (s, 1H)

^{31}P NMR ($CDCl_3$) δ : 153.32

Example 7 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl} adenine

15 ml of ethylenechloride and 15 ml of trifluoracetic acid were added to the compound (508 mg, 0.76 mmol) prepared from the step 10 of Example 2, and the resulting mixture was stirred at room temperature for 4 hours. After the reaction mixture was solidified under reduced pressure, this solid was purified by column chromatography eluted with 10%-25% methanol/methylene chloride to give 268 mg of the desired compound (95% yield).

^{1}H NMR (CD_3OD) δ 3.03 (s, 3H), 3.32 (m, 1H), 3.61 (dd, 1H, J=4.9, 12 Hz), 3.75 (dd, 1H, J=9.3, 9.3 Hz), 3.77 (dd, 1H, J= 12, 12 Hz), 3.88 (m, 2H), 4.07 (dd, 1H, J=9, 9 Hz), 4.75 (m, 1H), 7.50 (t, 1H, J=7.3 Hz), 7.61 (d, 1H, J=7 Hz), 8.48 (s, 1H), 8.68 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-

methyl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl}adenine

Methyliodide (42 μ L, 0.7 mmol), 4-dimethylamino pyridine (catalytic amount) and triethylamine (273 μ L, 1.75 mmol) were added to the title compound (140 mg, 0.35 mmol) prepared from step 1. The reaction mixture was stirred at room temperature for 15 hours, and concentrated under reduced pressure. The residue was dissolved in methylene chloride, the insoluble precipitate was removed by filtering, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography eluted with 10% - 25% methanol/methylene chloride to give 69 mg of the desired compound (48% yield).

^{1}H NMR (CD_3OD) δ 2.13 (m, 1H), 2.47 (s, 3H, N-Me), 3.10 (dd, 1H, J=5, 11 Hz), 3.17 (s, 3H, OMe), 3.22 (m, 1H), 3.72 (dd, 1H, J=9.5, 9.5 Hz), 3.95 (m, 2H), 3.99 (dd, 1H, J=9, 9 Hz), 4.67 (ddd, 1H, J=4.5, 11, 11 Hz), 7.59 (t, 2H, J=7.1 Hz), 7.67 (d, 1H, J=7.3 Hz), 8.11 (d, 2H, J=7.2 Hz), 8.54 (s, 1H), 8.74 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in

the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, N-Me), 2.36 (m, 1H), 2.96 (d, 1H, J=2.5 Hz), 3.02 (dd, 1H, J=4.4, 11.2 Hz), 3.11 (s, 3H, OMe), 3.22 (dd, 1H, J=11.4, 11.5 Hz), 3.45 (dd, 1H, J=4.1, 10.1 Hz), 3.55 (dd, 1H, J=2.3. 8.7 Hz), 3.80 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.97 (dd, 1H, J=8.6, 10.3 Hz), 4.56 (ddd, 1H, J=4.7, 11.3, 11.3 Hz), 6.88 (d, 4H, J=8.7 Hz), 7.17 - 7.65 (m, 12H), 8.00 (s, 1H), 8.05 (d, 2H, J=7.4 Hz), 8.84 (s, 1H).

(Step 4) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H, J=6.8 Hz), 1.08 (d, 3H, J=6.8 Hz), 2.32 (t, 2H, J=6.8 Hz), 2.76 (m, 1H), 2.91 (m, 1H), 3.00 (s, 3H, N-Me), 3.04 (m, 1H), 3.15 (dd, 1H, J=7.3, 10.1 Hz), 3.30 - 3.70 (m, 5H), 3.81 (s, 6H, OMe), 3.83 (m, 1H), 4.23 (dd, 1H, J=3.8, 5.7 Hz), 4.55 (ddd, 1H, J=4, 9.2, 9.2 Hz), 6.84 (d, 2H, J=8.8 Hz), 6.87 (d, 2H, J=8.8 Hz), 7.21 - 7.62 (m, 12H), 8.05 (d, 2H, J=7.3 Hz), 8.18 (s, 1H), 8.86 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 151.14, 151.84 (major)

Example 8 : Preparation of 6-N-benzoyl-{(3R, 4R, 5R, 6R)-N-propyl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}adenine

Under nitrogen atmosphere, the title compound prepared from the step 1 of Example 7 (240 mg, 0.6 mmol) was dissolved in 10 mL of anhydrous acetonitrile, to this resulting solution were added 4-dimethylaminopyridine (small amount), triethylamine (0.83 mL, 6 mmol) and n-propyliodide (300 μ L, 3 mmol). This reaction mixture was heated to reflux for 4 hours, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 7 - 25% methanol/dichloromethane (CH_2Cl_2:MeOH=10:1) in order to give trietylamine salt and 200 mg of the desired compound (45% yield).

^1H NMR ($CDCl_3$) δ 0.90 (t, 3H, J=7.2 Hz, N-$CH_2CH_2\underline{CH_3}$), 1.52 (m, 2H, N-$CH_2\underline{CH_2}CH_3$), 2.54 (m, 2H), 2.75 (m, 1H), 3.05 (s, 3H, OMe), 3.17 (m, 1H), 3.33 (dd, 1H,

J=11.4, 11.5 Hz), 3.83 (dd, 1H, J=9, 9 Hz), 3.90 - 4.10 (m, 3H), 4.50 (ddd, 1H, J=4.3, 10.6, 10.6 Hz), 7.54 (t, 2H, J=7.6 Hz), 7.62 (d, 1H, J=6.9 Hz), 8.05 (d, 2H, J=7.6 Hz), 8.05 (d, 2H, J=7.6 Hz), 8.06 (s, 1H), 8.82 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.67 (t, 3H, J=7.3 Hz, N-$CH_2CH_2\underline{C}H_3$), 1.32 (m, 2H, N-$CH_2\underline{C}H_2CH_3$), 2.30 (m, 1H), 2.48 (m, 1H), 2.64 (m, 1H), 3.05 (m, 1H), 3.10 (s, 3H, OMe), 3.23 (dd, 1H, J=11.3, 11.3 Hz), 3.45 (dd, 1H, 3.7, 10.1 Hz), 3.53 (dd, 1H, J=3.3, 10.1 Hz), 3.80 (s, 6H, OMe), 3.82 (m, 1H), 3.93 (dd, 1H, J=8.6, 10 Hz), 4.52 (ddd, 1H, J=4.3, 10.7, 10.7 Hz), 6.86 (d, 4H, J=8.7 Hz), 7.21 - 7.64 (m, 15H), 8.03 (s, 1H), 8.04 (d, 2H, J=8.5 Hz), 8.82 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from using title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J=7 Hz, N-$CH_2CH_2C\underline{H}_3$), 0.88 (t, 3H, J= 7 Hz, N-$CH_2CH_2C\underline{H}_3$), 0.94 (d, 6H, J=6.7 Hz, NCH($C\underline{H}_3$)$_2$)), 1.07 (d, 6H, J=6.7 Hz, NCH($C\underline{H}_3$)$_2$), 1.14 (d, 6H, J=6.8 Hz, NCH($C\underline{H}_3$)$_2$), 1.18 (d, 6H, J=6.8 Hz, NCH($C\underline{H}_3$)$_2$), 2.33 (t, 4H, J=6.3 Hz), 2.4 - 2.57 (m, 4H), 2.75 (t, 2H, J=6 Hz), 2.82 (m, 1H), 2.87 (m, 1H), 3.08 (m, 1H), 3.13 (m, 1H), 3.22 (s, 3H, OMe), 3.26 - 3.69 (m, 14H), 3.31(s, 3H, OMe), 3.81 (s, 6H, OMe), 4.23 (m, 3H), 4.29 (m, 1H), 4.33 (m, 1H), 4.63 (m, 1H), 4.74 (m, 2H), 6.84 (d, 4H, J=8.8 Hz), 6.85 (d, 4H, J=8.8 Hz), 7.21 - 7.64 (m, 24H), 8.05 (d, 2H, J=8.6 Hz), 8.64 (s, 1H), 8.80 (s, 1H), 8.81 (s, 1H), 8.92 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 149.94, 150.64

Example 9 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl}adenine

Under nitrogen atmosphere, the title compound

prepared from the step 1 of Example 7 (300 mg, 0.78 mmol) was dissolved in 10 mL of anhydrous acetonitrile, and to the resulting solution were added 4-dimethylaminopyridine (small amount), triethylamine (1.08 mL, 7,8 mmol) and benzylbromide (468 μ L, 3.9 mmol). This reaction mixture was heated to reflux for 4 hours, and solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 7-25% methanol/dichloro-methane (CH_2Cl_2 : MeOH = 15:1-4:1) to give 66 mg of the desired compound (18% yield).

^1H NMR ($CDCl_3$) δ 2.84 - 2.97 (m, 2H), 3.19 (dd, 1H, J=11.2, 11.2 Hz), 3.36 (m, 1H), 3.51 (s, 3H, OMe), 3.73 (d, 1H, J=9 Hz), 3.85 (ddd, 1H, 5, 9.2, 9.2 Hz), 3.99 (dd, 1H, J=9, 9 Hz), 4.09 (m, 2H), 4.32 (dd, 1H, J=6.7, 6.7 Hz), 7.23 - 7.45(m, 8H), 7.94(d, 2H, J=7.1 Hz), 8.04 (s, 1H), 8.41 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^1H NMR ($CDCl_3$) δ 2.70 (m, 1H), 2.98 (dd, 1H,

J=4.6, 11.6 Hz), 3.07 (s, 3H, OMe), 3.15 (m, 2H), 3.61 (m, 2H), 3.803 (s, 3H, OMe), 3.807 (s, 3H, OMe), 3.89 - 4.02 (m, 3H), 4.44 (ddd, 1H, J=4, 10, 10 Hz), 6.84 (d, 2H, J=8.93 Hz), 6.85 (d, 2H, J=8.9 Hz), 7.18 - 7.56 (m, 16H), 7.61 (d, 2H, J=7.2 Hz), 7.96(s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(cyanoethoxy) (N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3H, J=6.7 Hz), 1.08 (d, 3H, J=6.7 Hz), 1.12 (d, 3H, J=7 Hz), 1.14 (d, 3H, J=7 Hz), 2.37 (t, 1H, J=6.3 Hz), 2.43 (t, 1H, J=6.4 Hz), 2.80 (m, 1H), 3.34 (s, 2H, OMe), 3.10 - 3.79 (m, 10H), 3.81 (s, 3H, OMe), 3.82 (s, 3H, OMe), 4.32 (m, 1H), 4.58 (m, 1H), 4.73 (m, 1H), 6.84 (d, 4H, J=8.9 Hz), 7.21 - 7.64 (m, 18H), 8.04 (d, 2H, J=7.4 Hz), 8.42 (s, 1H), 8.82 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 150.53

Example 10 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropyl

amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

The title compound of the step 1 of Example 7 (450 mg, 1.17 mmol) was dissolved in 40 mL of anhydrous acetonitrile, and to the resulting solution were added α -bromo-p-tolunitrile (1 g, 5.85 mmol), triethylamine (1.62 mL, 10.7 mmol) and dimethylaminopyridine (small amount). This reaction mixture was stirred at room temperature for 14 hours, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography eluted with 5-25% methanol/methylene chloride to give 200 mg of the desired compound (32% yield).

^{1}H NMR ($CDCl_3$) δ 2.63 (m, 1H), 2.92 (dd, 1H, J=4.2, 11.3 Hz), 3.07 (s, 3H, OMe), 3.23 (m, 1H), 3.43 (d, 1H, J=14.2), 3.90 (dd, 1H, J=9, 9 Hz), 3.97 - 4.16 (m, 4H), 4.33 (d, 1H, J=14.3 Hz), 4.44 (ddd, 1H, J=4, 10.6, 10.6 Hz), 7.46 (m, 2H), 7.52 (d, 2H, J=7.7 Hz), 7.59 (d, 3H, J=8.3 Hz), 8.01 (d, 2H, J=9.8 Hz), 8.03 (s, 1H), 8.76 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-

(4-cyanobenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.75 (m, 1H), 2.86 (dd, 1H, J=4.5, 11.2 Hz), 3.07 (s, 3H, OMe), 3.20 (dd, 1H, J=5.4, 11 Hz), 3.30 (dd, 1H, J=5.7, 8 Hz), 3.57 (m, 2H), 3.788 (s, 3H, OMe), 3.794 (s, 3H, OMe), 3.88 (dd, 1H, J=9, 9Hz), 3.97 (m, 2H), 4.39 (ddd, 1H, J=4, 10, 10 Hz), 6.80 (d, 2H, J=8.7 Hz), 6.81 (d, 2H, J=8.7 Hz), 7.25 - 7.54 (m, 16H), 7.97(s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.80 (s, 1H).

(Step 3)Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.05 (d, 6H, J=6.7 Hz), 1.09 (d, 6H, J=6.2 Hz), 1.11 (d, 6H, J=5.6 Hz), 1.15 (d, 6H, J=6.7 Hz), 2.02 - 2.06 (m, 1H), 2.21 - 2.40 (m, 2H), 2.44 (t, 2H, J=6.1 Hz), 2.95 (m, 2H), 3.07 (s, 3H, OMe),

3.31 (s, 3H, OMe), 3.31 - 3.82 (m, 13H), 3.80 (s, 6H, OMe), 3.88 - 3.99 (m, 4H), 4.13 (m, 2H), 4.50 (m, 2H), 4.73 (m, 2H), 5.38 (m, 2H), 6.82 (d, 4H, J=8.6 Hz), 6.83 (d, 4H, J=8.1 Hz), 7.16 - 7.46 (m, 23H), 7.54 (t, 4H, J=8 Hz), 7.61 (d, 2H, J=8.9 Hz), 8.04 (m, 5H), 8.19 (s, 1H), 8.65 (s, 1H), 8.81 (s, 1H), 8.84 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 150.69, 151.45

Example 11 : Preparation of 6-N-benzoyl-{(3R,$R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound prepared from the step 1 of the Example and 4-fluorobenzylbromide via the procedure described in the step 1 of the Example 10.

^{1}H NMR ($CDCl_3$) δ 2.53 (m, 1H), 2.94 (dd, 1H, J=4.6 Hz), 3.2 (m, 2H), 3.94 (m, 2H), 4.02 (m, 2H), 4.15 (m, 2H), 4.42 (ddd, 1H, J=4.6, 10.4, 10.4 Hz), 7.46 (t, 2H, J=7.1 Hz), 7.52 (d, 2H, J=9.2 Hz), 7.64 - 7.54 (m, 3H), 7.97 (d, 2H, J=7.4 Hz), 8.01 (s, 1H), 8.72 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.69 (m, 1H), 2.93 (dd, 1H, J=4.5, 11.4 Hz), 3.06 (s, 3H, OMe), 3.14 (m, 1H), 3.35 - 3.28 (m, 2H), 3.59 (m, 2H), 3.80(s, 6H, OMe), 3.98 - 3.87 (m, 2H), 4.42 (ddd, 1H, J=4.6, 11.1, 11.1 Hz), 6.83 (d, 2H, J=6.5 Hz), 6.84 (d, 2H, J=7.1 Hz), 7.39 - 7.17 (m, 14H), 7.51 (t, 1H, J=7.0 Hz), 7.61 (d, 1H, J=7.5 Hz), 7.96 (s, 1H), 8.03 (d, 2H, J=7.2 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.99 (d, 6H, J=6.8 Hz), 1.06 (d, 6H, J=6.0 Hz), 1.09 (d, 6H, J=6.1 Hz), 1.14 (d, 6H,

J=6.8 Hz), 2.40 (m, 4H), 2.78 (m, 2H), 2.92 (m, 2H), 3.15 (s, 3H, OMe), 3.35 (s, 3H, OMe), 3.79 - 3.21 (m, 16H), 3.81 (s, 6H), 3.82 (s, 6H, OMe), 4.19 - 4.05 (m, 4H), 4.30 (m, 2H), 4.55 (m, 2H), 4.72 (m, 2H), 6.85 (d, 8H, J=8.8 Hz), 7.63 - 6.96 (m, 35H), 8.04 (d, 4H, J=8.2 Hz), 8.34 (s, 1H), 8.74 (s, 1H), 8.80 (s, 1H), 8.83 (s, 1H).

Example 12 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

(Step 1) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}adenine

The title compound prepared from the step 1 of Example 7 (200 mg, 0.5 mmol) was dissolved in 5 mL of anhydrous acetonitrile, to the resulting solution were added 4-methoxybenzylchloride (339 mg, 2.5 mmol), triethylamine (0.69 mL, 5 mmol) and dimethylamimo pyridine (small amount). This reaction mixture was heated to reflux for 2 hours, and concentrated under reduced pressure. The residue was purified by column chromatography eluted with 10-20% methanol/dichloromethane to give 104 mg of the desired compound (40 % yield).

^{1}H NMR ($CDCl_3$) δ 2.60 (d, 1H, J=9 Hz), 3.03 (s, 3H, OMe), 3.07 (m, 1H), 3.19 (dd, 1H, J=11.5, 11.5 Hz), 3.35 (d, 1H, J=13.4 Hz), 3.79 (s, 3H, OMe), 3.88 (dd, 1H, J=9, 9 Hz), 3.99 (dd, 1H, J=9, 9 Hz), 4.12 (m, 3H), 4.42 (ddd, 1H, J=4, 10.5, 10.5 Hz), 6.86 (d, 2H, J=8.6 Hz), 7.20 (d, 2H, J=8.6 Hz), 7.54 (t, 2H, J=7 Hz), 7.62 (d, 1H, J=7 Hz), 7.96 (s, 1H), 8.04 (d, 2H, J=7 Hz), 8.81 (s, 1H).

(Step 2) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.67 (m, 1H), 2.79 (m, 1H), 2.99 (dd, 1H, J=3, 9 Hz), 3.04 (m, 1H), 3.07 (s, 3H, OMe), 3.12 (m, 1H), 3.61 (m, 2H), 3.77 (s, 3H, OMe), 3.81 (s, 6H, OMe), 3.89 (d, 1H, J=9 Hz), 3.94 (m, 1H), 4.43 (ddd, 1H, J=4.7, 10, 10), 6.78 (d, 2H, J=8.7 Hz), 6.86 (d, 2H, J=8.6 Hz), 6.87 (d, 2H, J=8.7 Hz), 7.08 (d, 2H, J=8.5 Hz), 7.22~7.62 (m, 22H), 7.95 (s, 1H), 8.04 (d, 2H, J=7.4 Hz), 8.81 (s, 1H).

(Step 3) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-

(4-methoxybenzyl)-5-[(2-cyanoethoxy) (N,N-diisopropyl amino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H, J=6.7 Hz), 1.08 (d, 6H, J=6.7 Hz), 1.13 (d, 6H, J=6.7 Hz), 1.15 (d, 6H, J=6.7 Hz), 2.36 (t, 2H, J=6 Hz), 2.44 (t, 2H, J=6 Hz), 2.87 (dd, 2H, J= 4, 13 Hz), 3.10 (m, 4H), 3.19 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.77~3.36 (m, 12H), 3.79 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.82 (s, 6H, OMe), 4.33 (m, 1H), 4.58 (m, 1H), 4.72 (m, 1H), 6.85 (d, 4H, J=8.6 Hz), 6.88 (d, 4H, J=8.8 Hz), 7.50 - 7.11 (m, 13H), 7.53 (t, 2H, J=7.8 Hz), 7.61 (d, 1H, J=7.1 Hz), 8.05 (d, 2H, J=7.5 Hz), 8.45 (s, 2H), 8.77 (s, 2H).

^{31}P NMR ($CDCl_3$) δ : 150.41, 150.60

Example 13 : Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}cytosine

(Step 1) Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}cytosine

The title compound prepared from the step 7 of Example 1 (600 mg, 1.26 mmol) was dissolved in 30 mL of anhydrous dioxane, to this resulting solution were added the mixture of N^3-benzoylcytosine (600 mg, 1.26 mmol) and triphenylphosphine (1.5 g, 5.76 mmol). Diethylazodicarboxylate (1 mL, 1 mmol) dissolved in 12 mL of anhydrous tetrahydrofuran was added to this reaction mixture and stirred at room temperature overnight. Ethylacetate was added to the reaction mixture, and the resulting reaction mixture washed twice with 50 mL of distilled water. The organic layer was separated, water removed with sodium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel 60 column chromatography eluted with 50-75% ethylacetate/hexane (30 - 50% EtOAc/Hexane) to give 200 mg of the desired compound (24% yield).

^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H, Me), 2.64 (m, 1H), 3.00 (dd, 1H, J=4.0, 11.2 Hz), 3.59 (s, 3H, OMe), 3.70 (m, 2H), 3.82 (m, 2H), 3.88 (s, 3H, OMe), 4.23 (m, 1H), 4.46 (dd, 1H, J=3.8, 10.5 Hz), 5.01 (s, 1H), 6.98 (d, 2H, J=8.8 Hz), 7.69 - 7.12 (m, 17H), 7.92 (d, 2H, J=7.6 Hz).

(Step 2) Preparation of 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl-6-hydroxymethyl-5-hydroxy-4- methoxy

piperidine-3-yl}cytosine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.03 (m, 1H), 2.49 (m, 1H), 2.72 (m, 1H), 3.22 (dd, 1H, J=4,2, 12.0 Hz), 3.44 (s, 3H, OMe), 3.63 (m, 1H), 4.07 (m, 2H), 4.23 (dd, 1H, J=3.7, 12.3 Hz), 5.46 (s, 1H), 7.93 - 7.24 (m, 19H).

(Step 3) 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}cytosine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 2.52 (m, 1H), 2.58 (m, 1H), 3.09 (dd, 1H, J=3.8, 11.5 Hz), 3.41 (s, 3H, OMe), 3.62 (m, 1H), 3.77 (m, 2H), 3.79 (s, 3H, OMe), 3.80 (s, 3H, OMe), 4.17 (m, 2H), 5.04 (s, 1H), 6.80 (d, 2H, J=8.7 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.72 - 7.13 (m, 24H), 7.90 (d, 2H, J=7.4 Hz).

(Step 4) 4-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}

cytosine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.17 (d, 3H, J=6.8 Hz), 1.21 (d, 3H, J=6.7 Hz), 2.64 - 2.42 (m, 4H), 2.91 (dd, 1H, J=4.8, 13.3 Hz), 3.09 (m, 1H, OMe), 3.35 (s, 3H OMe), 3.64 - 3.48 (m, 5H), 3.829 (s, 3H OMe), 3.832 (s, 3H OMe), 4.19 (m, 1H), 4.48 (d, 1H, J=12.8 Hz), 4.64 (s, 1H), 4.68 (m, 1H), 5.04 (s, 1H), 6.82 (m, 4H), 7.71 - 7.14 (m, 2H), 7.91 (d, 2H, J=8.3 Hz).

^{31}P NMR ($CDCl_3$) δ : 149.72, 150.59

Example 14: Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}guanine

(Step 1) Preparation of 2-N-isobutyryl-6-O-[2-(p-nitrophenyl)ethyl]-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}guanine

The title compound prepared from the step 7 of Example 1 was dissolved in 70 mL of anhydrous xylene, to this resulting solution were added N^2-isobutyryl-O^6-[2-(p-nitrophenyl)ethyl]guanine (2.3 g, 6.2 mmol) and

triphenylphosphine (1.74 g, 6.6 mmol), and the reaction mixture was stirred at room temperature for 1 hour. The diethylcarboxylate (DEAD, 1.04 mL, 6.6 mmol) dissolved in 10 ml of anhydrous xylene, was slowly added to this reaction mixture for 20 min, and stirred at 120℃ for 6 hours. After slow cooling of the reaction mixture to room temperature, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with the mixed solvent of ethylacetate and hexane (1:1) to give 560 mg of the desired compound (22% yield).

^{1}H NMR ($CDCl_3$) δ 1.28 (d, 3H, J=4.9 Hz), 1.29 (d, 3H, J=5.3 Hz), 1.30 (d, 3H, J=4.6 Hz), 1.31 (d, 3H, J=5.3 Hz), 1.55 (s, 3H, Me), 1.81 (s, 3H, Me), 2.58 (m, 2H), 2.72 (dd, 2H, J=11.2, 11.3 Hz), 3.00 (dd, 1H, J=4.5, 11.2 Hz), 3.08 (m, 1H), 3.34 (t, 2H, J=6.9 Hz), 3.35 (s, 3H, OMe), 3.38 (s, 3H, OMe), 3.70 (d, 2H, J=9.5 Hz), 3.80 (d, 2H, J=9 Hz), 3.82 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.06 - 4.39 (m, 6H), 4.53 (m, 2H), 4.80 (m, 2H), 5.02 (s, 1H), 5.14 (s, 1H), 6.90 (d, 2H, J=8.9 Hz), 6.99 (d, 2H, J=8.8 Hz), 7.72 - 7.15 (m, 30H), 8.18 (d, 4H, J=8.7 Hz).

(Step 2) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)

ethylidene]piperidine-3-yl}guanine

The title compound of the step 1 was dissolved in 12 mL of anhydrous pyridine, and to this solution was added 1,8-diazabicyclo[5.4.0]undec-7-N (DBU, 203 mL, 1.35 mmol). The mixture was stirred at room temperature for 10 hours, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with 5-10% methanol/methylene chloride to give 375 mg of the desired compound (83% yield).

^{1}H NMR ($CDCl_3$) δ 1.310 (d, 3H, J=4.1 Hz), 1.32 (d, 3H, J=4 Hz), 1.33 (d, 3H, J=4.7 Hz), 1.34 (d, 3H, J=4.1 Hz), 1.56 (s, 3H, Me), 1.80 (s, 3H, Me), 2.51 (dd, 1H, J=11.2, 11.3 Hz), 2.62 (m, 3H), 3.02 (dd, 1H, J=4.5, 11.2 Hz), 3.08 (m, 1H), 3.40 (s, 3H, OMe), 3.45 (s, 3H, OMe), 3.69 (d, 2H, J=9.5 Hz), 3.77 (d, 2H, J=9 Hz), 3.82 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.27 - 4.05 (m, 6H), 4.58 - 4.45 (m, 2H), 5.02 (s, 1H), 5.13 (s, 1H), 6.90 (d, 2H, J=8.7 Hz), 6.99 (d, 2H, J=8.9 Hz), 7.72 - 7.15 (m, 26H).

(Step 3) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-6-hydroxymethyl-5-hydroxy-4-methoxy piperidine-3-yl}guanine

This compound was prepared from the title compound of the step 2 via the procedure described in

the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.25 (d, 3H, J=7 Hz), 1.28 (d, 3H, J=7 Hz), 2.59 (d, 1H, J=8.5 Hz), 2.71 (dd, 1H, J=10.5, 10.5 Hz), 2.87 (dq, 1H, J=7.7 Hz), 3.07 (m, 1H), 3.16 (s, 3H, OMe), 3.65 (dd, 1H, J=9, 9 Hz), 4.06 (dd, 1H, J=8.6, 8.6 Hz), 4.21 (m, 2H), 4.41 (m, 1H), 5.56 (s, 1H), 7.30 - 7.12 (m, 10H), 7.86 (s, 1H).

(Step 4) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}guanine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3H, J=7.2 Hz), 1.30 (d, 3H, J=6.8 Hz), 2.56 (d, 1H, J=8.7 Hz), 2.66 (m, 2H), 3.08 (dd, 1H, J=4.2, 11.4 Hz), 3.15 (s, 3H, OMe), 3.29 (dq, 1H, J=1.5, 5.7 Hz), 3.57 (dd, 1H, J=8.9, 8.9 Hz), 3.67 (m, 1H), 3.77 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.81 (m, 1H), 4.39 (ddd, 1H, J=4, 11.6, 11.6 Hz), 5.06 (s, 1H), 6.80 (d, 2H, J=8.9 Hz), 6.81 (d, 2H, J=8.8 Hz), 7.48 - 7.22 (m, 15H), 7.65 (s, 1H).

(Step 5) Preparation of 2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)

phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}guanine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.15 (d, 6H, J=6.7 Hz), 1.16 (d, 6H, J=7.3 Hz), 1.19 (d, 6H, J=6.5 Hz), 1.21 (d, 6H, J=6.7 Hz), 1.26 (d, 6H, J=6.8 Hz), 1.27 (d, 6H, J=6.8 Hz), 2.38 (t, 4H, J=7 Hz), 2.94 - 2.42 (m, 10H), 3.18 (s, 3H, OMe), 3.24 (s, 3H, OMe), 3.30 (dd, 1H, J=5.7, 7 Hz), 3.40 (m, 1H), 3.80 - 3.53 (m, 10H), 3.812 (s, 3H, OMe), 3.818 (s, 3H, OMe), 4.17 (m, 1H), 4.53 - 4.37 (m, 3H), 4.67 (s, 1H), 4.72 (s, 1H), 6.81 (d, 2H, J=8.8 Hz), 6.83 (d, 2H, J=8.9 Hz), 7.13 - 7.35 (m), 8.32 (s, 1H), 8.63 (s, 1H).

^{31}P NMR ($CDCl_3$) δ : 149.32, 150.08

Example 15 : Preparation of {(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

(Step 1) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl)ethylidene]piperidine-3-yl}thymine

The title compound prepared from the step 7 of

Example 1 (2.2 g, 4.63 mmol) was dissolved in 100 mL of anhydrous dioxane, to this resulting solution were added N^3-benzoylthymine (2.7 g, 20.73 mmol) and triphenylphosphine (3.3 g, 12.58 mmol), and stirred. To this reaction mixture was added diethyl azodicarboxylate (DEAD, 2.1 mL, 11.87 mmol) dissolved in 15 mL of anhydrous tetrahydrofuran, and the resulting reaction mixture was stirred at room temperature for 12 hours. The reaction mixture was concentration under reduced pressure, the residue was dissolved in 90 mL of methanol, and saturated with ammonia gas for 30 min. The saturated residue was concentrated under reduced pressure with adding toluene, and purified by silica gel column chromatography eluted with 30-50% ethylacetate/hexane in order to give 2.45 g of the desired compound (95% yield).

^{1}H NMR ($CDCl_3$) δ 1.79 (s, 3H, Me), 1.89 (s, 3H, Me), 2.54 (ddd, 1H, J=4, 10, 10 Hz), 3.01 (dd, 1H, J=4.3, 11.1 Hz), 3.56 (s, 3H, OMe), 3.45 (s, 3H, OMe), 3.62 (m, 1H), 3.82 (s, 3H, OMe), 4.12 (dd, 1H, J=7.1, 7.2 Hz), 4.15 (m, 3H), 4.52 (dd, 1H, J=4.2, 10.7 Hz), 5.09 (s, 1H), 6.86 (s, 1H, vinyl H), 6.89 (d, 2H, J=8.9 Hz), 7.12 - 7.47 (m, 12H).

(Step 2) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-6-hydroxymethyl-5-hydroxy-4-methoxypiperidine-3-yl}

thymine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 3.35 (s, 3H, OMe), 1.89 (s, 3H, Me), 2.53 (dd, 1H, J=8.3, 12.5 Hz), 3.08 (dd, 1H, J=4, 11.4 Hz), 3.33 (m, 1H), 3.35 (s, 3H, OMe), 3.54 (s, 3H, OMe), 3.77 (dd, 1H, J=8.4, 8.4 Hz), 3.97 (dd, 1H, J=8.2, 8.3 Hz), 4.10 (d, 1H, J=10.5 Hz), 4.22 (m, 2H), 5.46 (s, 1H), 7.05 (s, 1H, vinyl H), 7.20 - 7.41 (m, 10H).

(Step 3) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H, Me), 2.47 (d, 1H, J=9 Hz), 2.48 (m, 1H), 3.03 (dd, 1H, J=4, 10.5 Hz), 3.44 (s, 3H, OMe), 3.53 - 3.79 (m, 5H), 3.791 (s, 3H, OMe), 3.797 (s, 3H, OMe), 5.05 (s, 1H), 6.80 (d, 4H, J=8.8 Hz), 6.81 (d, 4H, J=8.8 Hz), 6.83 (s, 1H, vinyl H), 7.22 - 7.37 (m, 15H), 7.47 (d, 2H, J=6.8 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-5-

[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.27 (d, 6H, J=6.7 Hz), 1.29 (d, 6H, J=6.8 Hz), 1.91 (s, 3H, Me), 2.49 (d, 1H, J=8.5 Hz), 2.57 (d, 1H, J=3.1 Hz), 2.77 (t, 2H, J=6.2 Hz), 3.03 (dd, 1H, J=4.2, 11.4 Hz), 3.44 (s, 3H, OMe), 3.46 - 3.73 (m, 6H), 3.76 (m, 1H), 3.790 (s, 3H, OMe), 3.796 (s, 3H, OMe), 4.13 (m, 2H), 5.05 (s, 1H), 6.80 (d, 2H, J=8.8 Hz), 6.81 (d, 2H, J=8.9 Hz), 7.00 (s, 1H, vinyl H), 7.20 - 7.37 (m, 15H), 7.46 (d, 2H, J=7 Hz).

Example 16 : Preparation of 3-N-methyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy) (N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxy piperidine-3-yl}thymine

(Step 1) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-methoxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}thymine

The title compound prepared from the step 7 of Example 1 (1.4 g, 2.94 mmol) was dissolved in 65 mL of anhydrous dioxane, to this solution were added N^3-

benzoylthymine (1.7 g, 13.1 mmol) and triphenylphosphine (2.1 g, 8 mmol), and this resulting solution was stirred. To this reaction mixture was added diethyl azodicarboxylate (DEAD, 1.34 mL, 1.8 mmol) dissolved in 10 mL of anhydrous tetrahydrofuran, and stirred at room temperature for 12 hours. The reaction mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography eluted with 30% ethylacetate/hexane in order to give 1.9 g of the desired compound (94% yield).

^{1}H NMR ($CDCl_3$) δ 1.78 (s, 3H, Me), 1.94 (s, 3H, Me), 2.54 (m, 1H), 3.04 (dd, 1H, J=3.3, 10 Hz), 3.62 (s, 3H, OMe), 3.68 (m, 1H), 3.82 (s, 3H, OMe), 4.01 (dd, 1H, J=10, 10 Hz), 4.13 - 4.32 (m, 3H), 4.51 (dd, 1H, J=7, 10.5 Hz), 5.08 (s, 1H), 6.89 (s, 2H, J=8.8 Hz), 6.96 (s, 1H, vinyl H), 7.13 - 7.51 (m, 12H), 7.64 (d, 1H, J=7.4 Hz), 7.91 (d, 2H, J=7.2 Hz).

(Step 2) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-6-hydroxymethyl-5-hydroxy-4- methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 1 of the Example 7.

^{1}H NMR (CD_3OD) δ 1.99 (s, 3H, Me), 3.19 (ddd, 1H,

J=4.2, 4, 10.3 Hz), 3.32 (m, 2H), 3.58 (m, 1H), 3.60 (s, 3H, OMe), 3.74 (dd, 1H, J=10.4, 10.4 Hz), 3.89 (m, 1H), 3.91 (m, 2H), 7.50 (t, 1H, J=7.6 Hz), 7.74 (s, 1H), 7.75 (d, 1H, J=7.5 Hz), 7.96 (d, 2H, J=7.2 Hz).

(Step 3) Preparation of 3-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 2 of the Example 7.

^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H, Me), 2.02 (m, 1H), 2.36 (s, 3H, NMe), 2.80 (m, 1H), 2.99 (dd, 1H, J=4, 4, 10.8 Hz), 3.51 (s, 3H, OMe), 3.85 - 3.72 (m, 4H), 3.96 (d, 1H, J=11 Hz), 7.13 (s, 1H, vinyl H), 7.50 (t, 1H, J=7.5 Hz), 7.65 (d, 1H, J=7.4 Hz), 7.92 (d, 2H, J=7.2 Hz).

(Step 4) Preparation of [(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl} thymine

The title compound of the above step 3 (50 mg, 0.12 mmol) was dissolved in 5 mL of methanol, saturated at 0℃ with ammonia gas for 10 min, and stirred at room temperature for 10 min. The oversaturated ammonia gas was removed through stirring of the open reaction

system. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography eluted with 10 - 25% methanol/methylene chloride to give 26 mg of the desired compound (73% yield).

^{1}H NMR (CD_3OD) δ 1.91 (s, 3H, Me), 1.95 (m, 1H), 2.40 (s, 3H, NMe), 2.60 (m, 1H), 2.88 (dd, 1H, J=4.4, 11 Hz), 3.44 (m, 1H), 3.46 (s, 3H, OMe), 3.61 (m, 2H), 3.89 (d, 2H, J=2.5 Hz), 7.59 (s, 1H, vinyl H).

(Step 5) Preparation of {(3R,4R,5R,6R)-N-methyl-5-hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H, Me), 2.14 (s, 3H, NMe), 2.17 (m, 1H), 2.65 (m, 1H), 2.86 (dd, 1H, J=4.2, 11 Hz), 3.42 (m, 1H), 3.45 (s, 3H, OMe), 3.48 (m, 1H), 3.65 (m, 1H), 3.77 (m, 1H), 3.80 (s, 6H, OMe), 4.23 (dd, 1H, J=3.8, 5.8 Hz), 6.49 (d, 2H, J=5.1 Hz), 6.50 (d, 2H, J=5 Hz), 7.01 (s, 1H, vinyl H), 7.48 - 7.22 (m, 9H).

(Step 6) Preparation of {(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-

dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.11 (d, 6H, J=6.5 Hz), 1.12 (d, 12H, J=6.7 Hz), 1.16 (d, 6H, J=6.8 Hz), 1.93 (s, 6H, Me), 2.30 (s, 3H, NMe), 2.34 (s, 3H, NMe), 2.55 (t, 4H, J=6 Hz), 2.79 - 2.63 (m, 3H), 2.86 (m, 3H), 3.11 (dd, 1H, J=7, 10 Hz), 3.23 (dd, 1H, J=5.6, 10 Hz), 3.33 (s, 3H, OMe), 3.34 (s, 3H, OMe), 3.74 - 3.38 (m, 15H), 3.81 (s, 12H, OMe), 4.15 (m, 2H), 4.20 (m, 2H), 4.38 (m, 1H), 6.86 - 6.82 (m, 8H), 7.47 - 7.28 (m, 20H).

^{31}P NMR ($CDCl_3$) δ : 151.73, 151.90

Example 17 : Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

(Step 1) Preparation of {(3R,4R,5R,6R)-5-hydroxy-6-hydroxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound prepared from the step 1 of the Example 15 via the procedure described in the step 1 of Example 7.

^{1}H NMR (DMSO) δ 1.80 (s, 3H, Me), 3.16 (m, 1H), 3.28 (m, 2H) 3.33 (s, 3H, OMe), 3.53 - 3.66 (m, 4H), 3.78 (m, 1H), 5.47 (s, 1H), 7.74 (s, 1H, vinyl H).

(Step 2) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-hydroxy-6-dimethyltrityloxymethylhydroxymethyl-4-methoxypiperidine-3-yl}thymine

The title compound of the above step 1 (800 mg, 2.8 mmol) was dissolved in 18 mL of dioxane, and to this resulting mixture were added 9-fluorenylmethoxycarbonyl chloride (1.04 g, 2.32 mmol) and 35 mL of 10 % sodium carbonate solution at 0℃. After stirring of this reaction mixture at 0℃ for 1 hour, this reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluted with 50% ethylacetate/hexane to give 600 mg of the desired compound (42% yield).

^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H, Me), 3.37 (m, 1H), 3.46 (s, 3H, OMe), 3.50 (m, 5H), 3.89 (m, 2H), 4.25 (dd, 1H, J=5.4, 5.4 Hz), 4.55 (dd, 1H, J=5.3, 10.5 Hz), 4.70 (dd, 1H, J=5.5, 10.5 Hz), 6.85 (s, 1H, vinyl H), 7.33 (t, 2H, J=7.3 Hz), 7.42 (t, 2H, J=7.3 Hz), 7.57 (dd, 2H, J=4, 7.3 Hz), 7.77 (d, 2H, J=7.5 Hz).

(Step 3) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-

hydroxy-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3H, Me), 3.40 (m, 2H), 3.41 (s, 3H, OMe), 3.58 (m, 1H), 3.761 (s, 3H, OMe), 3.763 (s, 3H, OMe), 3.94 - 4.17 (m, 4H), 4.26 (dd, 1H, J=6.3, 10.5 Hz), 4.63 (m, 2H), 6.89 (d, 4H, J=9 Hz), 7.03 (s, 1H, vinyl H), 7.22 - 7.77 (m, 15H), 8.24 (dd, 2H, J=1.5, 5 Hz).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} thymine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 13 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.04 (d, 6H, J=6.5 Hz), 1.09 (d, 6H, J=6.7 Hz), 1.15 (d, 12H, J=6.3 Hz), 1.79 (s, 3H, Me), 1.81 (s, 3H, Me), 2.31 (t, 4H, J=6 Hz), 2.64 (m, 2H), 1.93 (dd, 1H, J=5, 15 Hz), 3.26 (s, 3H, OMe), 3.43 (s, 3H, OMe), 3.37 - 3.54 (m, 8H), 3.58 - 3.65 (m, 4H), 3.71 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.78 (s, 6H, OMe),

4.11 - 4.32 (m, 9H), 4.43 (m, 2H), 4.62 (m, 1H), 4.74 (m, 3H), 6.78 (m, 8H), 7.22 - 7.48 (m, 26H), 7.55 (d, 2H, J=7.9 Hz), 7.64 (d, 2H, J=8.3 Hz), 7.70 - 7.83 (m, 5H).

^{31}P NMR ($CDCl_3$) δ : 150.02, 150.84

Example 18 : Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxy piperidine-3-yl}adenine

(Step 1) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-*t*-butyldimethylsilyloxy-4-isobutyryloxy-5,6-O-[(4-methoxy phenyl)ethylidene]piperidine

As a starting material, diastereomer B prepared from the step of Example 1 (or diastereomer A or mixture of A and B), was dissolved in 100 mL of anhydrous pyridine, to this resulting mixture were added triethylamine (2.2 mL, 20.74 mmol) and isobutyric anhydride (5.8 mL, 26.62 mmol), and this reaction mixture was stirred at 60℃ for 1 day. The reaction mixture was cooled at room temperature, to the cooled reaction mixture was added 7 mL of methanol, and stirred for 30 min. The solvent was evaporated under reduced pressure, the residue diluted with ethylacetate, and washed with saturated sodium bicarbonate solution. The organic layer was separated, dried with sodium

sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography eluted with hexane/ethylacetate (10:1) to give 2.45 mg of the desired compound (55% yield).

^{1}H NMR ($CDCl_3$) δ -0.14 (s, 3H, Si-Me), -0.04 (s, 3H, Si-Me), 0.76 (s, 9H, Si-tBu), 1.27 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.35 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.54 (s, 3H, Me), 2.01 (dd, 1H, J=10.8, 10.8 Hz), 2.56 (ddd, 1H, J=4, 10.4, 10.4 Hz), 2.68 (m, 1H), 2.87 (dd, 1H, J=4.9, 11.4 Hz), 3.56 (d, 1H, J=8.4 Hz), 3.61 (d, 1H, J=6.9 Hz), 3.67 (dd, 1H, J=4.8, 10 Hz), 3.81 (s, 3H, OMe), 4.38 (dd, 1H, J=4, 10.4 Hz), 4.90 (dd, 1H, J=9.4, 9.4 Hz), 4.94 (s, 1H), 6.95 (d, 2H, J=8.7 Hz), 7.14 - 7.40 (m, 12H).

(Step 2) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-hydroxyl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 1 via the procedure described in the step 7 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.32 (d, 3H, J=6.4 Hz, $CH(CH_3)_2$), 1.34 (d, 3H, J=6.6 Hz, $CH(CH_3)_2$), 1.49 (s, 3H, Me), 1.96 (dd, 1H, J=10.9, 11 Hz), 2.55 (m, 1H), 2.77 (m, 1H), 3.08 (dd, 1H, J=5.1, 11.5 Hz), 3.52 - 3.80 (m, 3H),

3.87 (s, 3H, OMe), 4.47 (dd, 1H, J=4.1, 10.4 Hz), 4.67 (dd, 1H, J=9.2, 9.2 Hz), 4.97 (m, 1H), 6.97 (d, 2H, J=8.7 Hz), 7.14 - 7.44 (m, 12H).

(Step 3) Preparation of (3R,4R,5R,6R)-N-benzhydryl-3-methansulfonyl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine

This compound was prepared from the title compound of the step 2 via the procedure described in the step 8 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.35 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.48 (s, 3H, Me), 2.17 (dd, 1H, J=10.8, 10.8 Hz), 2.57 (ddd, 1H, J=4, 10.2, 10.2 Hz), 2.74 (m, 1H), 2.90 (s, 3H, OSO_2CH_3), 3.22 (dd, 1H, J=5.2, 11.1 Hz), 3.62 (dd, 1H, J=10.4, 10.5 Hz), 3.68 (dd, 1H, J=9.2, 9.3 Hz), 3.87 (s, 3H, OMe), 4.45 (dd, 1H, J=4.2, 10.6 Hz), 4.68 (ddd, 1H, J=5.2, 5.3, 10.5 Hz), 4.98 (s, 1H), 5.06 (dd, 1H, J=9.4, 9.6 Hz), 6.96 (d, 2H, J=8.9 Hz), 7.15 - 7.42 (m, 12H).

(Step 4) Preparation of {(3R,4R,5R,6R)-N-benzhydryl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl)ethylidene] piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 3 via the procedure described in the step 9 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.97 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 1.50 (s, 3H, Me), 2.40 (m, 1H), 2.57 (dd, 1H, J=11.4, 11.6 Hz), 2.77 (m, 1H), 3.11 (dd, 1H, J=4.4, 11.3 Hz), 3.72 (dd, 1H, J=10.4, 10.4 Hz), 3.89 (s, 3H, OMe), 3.92 (m, 1H), 4.53 (dd, 1H, J=4, 10.5 Hz), 4.82 (ddd, 1H, J=4.3, 11.2, 11.3 Hz), 5.06 (s, 1H), 5.42 (dd, 1H, J=9.7, 10.4 Hz), 6.97 (d, 2H, J=8.7 Hz), 7.13 - 7.75 (m, 12H), 7.75 (s, 1H), 8.33 (s, 1H).

(Step 5) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-4-isobutyryloxy-5,6-O-[(4-methoxyphenyl) ethylidene]piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 4 via the procedure described in the step 10 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.97 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 1.51 (s, 3H, Me), 2.40 (m, 1H), 2.62 (dd, 1H, J=11.3, 11.4 Hz), 2.80 (m, 1H), 3.15 (dd, 1H, J=4.4, 11.3 Hz), 3.74 (dd, 1H, J=10.4, 10.5 Hz), 3.89 (s, 3H, OMe), 3.94 (dd, 1H, J=9.2, 9.2 Hz), 4.54 (dd, 1H, J=4, 10.5 Hz), 4.91 (m, 1H), 5.08 (s, 1H), 5.48 (dd, 1H, J=10, 10 Hz), 6.97 (d, 2H, J=8.8 Hz), 7.13 - 7.61 (m, 15H), 7.97 (s, 1H), 8.02 (d, 1H, J=7.3 Hz), 8.79 (s, 1H).

(Step 6) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-hydroxymethyl-4-isobutyryloxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 5 via the procedure described in the step 11 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.75 (d, 3H, J=7 Hz, $CH(CH_3)_2$), 0.90 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 2.33 (m, 1H), 2.71 - 2.76 (m, 2H), 3.26 (dd, 1H, J=4.1, 11.6 Hz), 4.14 - 4.23 (m, 2H), 4.34 (m, 1H), 4.91 (ddd, 1H, J=3.8, 11, 11 Hz), 5.37 (dd, 1H, J=9.2, 10.6 Hz), 5.62 (s, 1H), 7.17 - 7.40 (m, 10H), 7.52 (t, 2H, J=7.2 Hz), 7.60 (d, 1H, J=7.2 Hz), 7.90 (s, 1H), 8.02 (d, 1H, J=7.7 Hz), 8.79 (s, 1H).

(Step 7) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-1N-benzhydryl-5-hydroxy-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine

This compound was prepared from the title compound of the step 6 via the procedure described in the step 12 of the Example 1.

^{1}H NMR ($CDCl_3$) δ 0.74 (d, 3H, J=7.1 Hz, $CH(CH_3)_2$), 0.91 (d, 3H, J=6.9 Hz, $CH(CH_3)_2$), 2.34 (m, 1H), 2.63 (d, 1H, J=8.9 Hz), 2.75 (dd, 1H, J=11.3, 11.3 Hz), 3.25 (dd,

1H, J=4.2, 11.4 Hz), 3.79 (s, 6H, OMe), 4.23 (ddd, 1H, J=4.1, 10.8, 10.8 Hz), 5.20 (s, 1H), 5.33 (dd, 1H, J=9.5, 10.2 Hz), 6.81 (d, 2H, J=8.9 Hz), 6.83 (d, 2H, J=9 Hz), 7.22 - 7.41 (m, 19H), 7.52 (t, 2H, J=6.9 Hz), 7.59 (d, 1H, J=7.1 Hz), 7.98 (s, 1H), 8.02 (d, 1H, J=7.5 Hz), 8.81 (s, 1H).

(Step 8) Preparation of 6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino) phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxy piperidine-3-yl}adenine

This compound was prepared from the title compound of the step 7 via the procedure described in the step 13 of the Example 1.

^1H NMR ($CDCl_3$) δ 0.83 (d, 3H, J=7.3 Hz, $COCH(CH_3)_2$), 0.92 (d, 3H, J=6.9 Hz, $COCH(CH_3)_2$), 1.09 (d, 6H, J=6.7 Hz, $NCH(CH_3)_2$), 1.17 (d, 6H, J=6.9 Hz, $NCH(CH_3)_2$), 2.22 (m, 1H), 2.44 (t, 2H, J=6.5 Hz), 2.78 (dd, 1H, J=6.5, 12.5 Hz), 3.06 (dd, 1H, J=4.1, 12.6 Hz), 3.45 - 3.60 (m, 3H), 3.62 - 3.88 (m, 4H), 3.80 (s, 3H, OMe), 3.81 (s, 3H, OMe), 4.48 (m, 1H), 4.77 (s, 1H), 4.82 (m, 1H), 5.40 (t, 1H, J=5.4 Hz), 6.79 (d, 2H, J=8.8 Hz), 6.80 (d, 2H, J=8.8 Hz), 7.11 - 7.56 (m, 22H),8.05 (d, 2H, J=7.2 Hz), 8.71 (s, 1H), 8.76 (s, 1H).

^{31}P NMR ($CDCl_3$) δ 150.56, 150.83.

Example 19 : The synthesis of oligomers

All oligomers was synthesized trityl-on with an Applied Biosystem 392 (DNA/RNA synthesizer) (1 μ mol scale). Time of general condensation is 1 min, whereas was 10 min in case of nucleotides containing azasugar having other group except for hydrogen at position 4. Solid support and protective group were removed by heating with ammonium hydroxide for 17 hours at 55℃, and this solution was freeze-dried by adding 5 drops of triethylamine every hour in order to inhibit deprotecting of the protective group. The residue was dissolved in 1 mL of 100 mM triethylammonium acetate (TEAA) at pH 7, and purified by reversed phase high performance liquid chromatography (RP-HPLC, Hamilton PRP-1, 300 mm X 7 mm, 18 - 28% acetonitrile/100 mM TEAA, pH 7, monitored at 260 nm). The desired fraction was freeze-dried, the residual TEAA was removed through adding twice 1 mL of distilled water, and freeze-dried. The residual solid was thoroughly dissolved by vortexing in 0.3 mL of 80% acetic acid, and dimethoxytrityl group was removed by incubating at room temperature for 20 min. 0.3 mL of Ethanol was added to the above solution to remove acetic acid, and freeze-dried. 1 mL of Distilled water was added to the residue, and dissolved by vortexing. 1 mL of Ether was added to the resulting solution, and stirred well by

vortexing. Ether layer was removed by using pipet, 1 mL of ether was again added, and stirred well. This procedure was repeated twice. After freeze-drying of water layer, 1 mL of distilled water was added to the residue, and the resulting solution was quantified by UV absorbance at 260 nm at 70℃. The extinction coefficients (at 260 nm) of natural nucleotides used for calculations were as follows: dAMP : 15200; dCMP : 7700; TMP : 8830; dGMP : 11500. The extinction coefficients of nucleotides having azasugar were considered as the same value as that of natural nucleotides. All oligomers were characterized by enzyme digestion followed by HPLC (Hewlett Packard, ODS hypersil, C-18; 20 mM K_2HPO_4, pH 5.6 (A), MeOH (B), 100% A to 40% B, 20 min) and laser desorption mass spectrometry.

Example 20 : Hybridization properties of oligomer : melting studies

UV absorbance versus temperature profiles was measured on a Beckmann DU 650 spectrophotometer with Beckmann high performance temperature controller. Nitrogen gas was passed over the cell at less than room temperature to avoid the condensation of moisture. The temperature of the cell holder was increased from 5℃ to 90℃ in 1℃ increments at a heating rate of 1℃/min.

2.5 μM of antisense oligomer and RNA, and buffer (100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7) were employed. Melting temperature (Tm) was determined by first derivative of absorbance versus temperature curve. Reverse melting temperature was also measured (90℃ to 5℃, heating rate of 1℃/min), and found to give reverse Tm within ± 1℃ of forward Tm. Tm of DNA-RNA was compared with Tm of RNA complementary with azasugar-containing antisense oligomer. High Tm indicated strong binding affinity.

<Table 1>Tm of antisense oligomers

	R^1	R^2	Sequence	Tm (RNA)	Δ Tm
Control	-	-	5'-dAGG GAG AGA AAG-3' 5'-rCTT TCT CTC CCT-3'	34℃	-
Example 1	β-OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	36℃	+2
			5'-AGG G**A**G AG**A** AAG-3'	39℃	+5℃
			5'-**A**GG G**A**G AGA AAG-3'	40℃	+6℃
Example 2	β-OMt	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	36℃	+2℃
			5'-AGG G**A**G AG**A** AAG-3'	38℃	+4℃
Example 3	β-OMt OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3	37℃	+1℃
Example 4	H	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	34℃	0℃
			5'-AGG G**A**G AG**A** AAG-3'	34℃	0℃
Example 5	α-OMe	Benzhydryl	5'-AGG G**A**G AGA AAG-3'	36℃	+2℃

	OMe		5'-AGG GAG AGA AAG-3'	37℃	3℃
Example 6	β - OMe	Benzhydryl	5'-AGG GAG AGA AAG-3'	33℃	-1℃
			5'-AGG GAG AGA AAG-3'	34℃	0℃
Example 7	β - OMe	Me	5'-AGG GAG AGA AAG-3'	28℃	-6℃
			5'-AGG GAG AGA AAG-3'	20℃	-14℃
Example 8	β - OMe	n-propyl	5'-AGG GAG AGA AAG-3'	31℃	-3℃
			5'-AGG GAG AGA AAG-3'	26℃	-8℃
Example 9	β - OMe	Benzyl	5'-AGG GAG AGA AAG-3'	33℃	-1℃
			5'-AGG GAG AGA AAG-3'	33℃	-1℃
Example 10	β - OMe	4-cyanobenzyl	5'-AGG GAG AGA AAG-3'	32℃	-2℃
			5'-AGG GAG AGA AAG-3'	31℃	-3℃
Example 11	β - OMe	4-fluoro-benzyl	5'-AGG GAG AGA AAG-3'	33℃	-1℃
			5'-AGG GAG AGA AAG-3'	33℃	-1℃
Example 12	β - OMe	4-methoxy-benzyl	5'-AGG GAG AGA AAG-3'	31℃	-3℃
			5'-AGG GAG AGA AAG-3'	31℃	-3℃
Example 14	β - OMe	Benzhydryl	5'-AGG GAG AGA AAG-3'	25℃	-9℃
			5'-AGG GAG AGA AAG-3'	20℃	-14℃
Example 18	β -OH	Benzhydryl	5'-AGG GAG AGA AAG-3'	31℃	-3℃

<Table 2> Tm of antisense oligomer

	R1	R2	Sequence	Tm (RNA)	Δ Tm
Control	-	-	5'-rAGG GAG AGA AAG-3' 5'-dCTT TCT CTC CCT-3'	54℃	-
Example 13	β -OMe	Benz-	5'-CTT TCT CTC CCT-3'	34℃	-

13		hydryl			20℃
Example 15	β -OMe	Benz-hydryl	5'-CTT TCT C<u>T</u>C CCT-3'	46℃	-8℃
Example 16	β -OMe	Me	5'-CTT TCT C<u>T</u>C CCT-3'	42℃	-12℃
Example 17	β -OMe-	H(Fmoc protec-ting group)	5'-CTT TCT C<u>T</u>C CCT-3	40℃	-14℃

As indicated in Table 1 and 2, all oligomers showed strong binding affinities for RNA than natural type DNA helix, especially 1-6℃ increment in Tm of the oligomers having monomers of Example 1, 2, 3 or 5, indicated that the oligomers bind with greatest affinity to a complementary strand of RNA.

Thus, the oligomers of the present invention can be used as antisense oligomers with great binding affinity for RNA.

Example 21 : Stability of oligomers against nuclease.

Into an eppendorf tube were added 0.15 OD (optical density) of each oligomer, 0.03 units of snake vernom diesterase and 0.18 units of alkaline phosphatase (Buffer : 100 mM $MgCl_2$, 4 μ L: 0.25 M Tris HCl, pH 8.1, 8 μ L: distilled water 13 μ L). The reaction mixture was incubated at 37℃, and aliquots were removed every 30 min. The amount of oligomer degradation associated with each aliquot was determined by HPLC (Hamilton PRP-1, 300 mm x 7 mm, 260 nm). Under

these conditions, all of the natural oligomers were degraded within 30 min, whereas the three oligomers of Example 1 were not completely degraded even after 4 hours. The monomers of the present invention did not show its peak on HPLC except for segments of oligomers partially degraded. This result demonstrated that the above nucleases did not degrade DNA segments linked to the monomers of the present invention.

Example 22 : Acute toxicity test with rat via perental route

Specific pathogen-free (SPF) SD-rats which were six weeks old, were tested for acute toxicity. Suspensions of the compounds of the Example 1 - 19 in 0.5% methyl cellulose were orally administered once at a dose of 1 g/kg/15ml to the rats, which were grouped in twos. After the administration, the animals were observed as to their death, clinical symptoms and weight change, and serological and serobiochemically tested. An autopsy was made over the rats with the naked eye to observe whether their abdominal and thoracic organs were damaged. Neither sudden death nor noticeable clinical symptoms were detected from all of the animals administered with the compounds of interest. In addition, no toxic signs were observed in weight change, serologic test, serobiochemical test,

and corpse examination. The compounds tested caused no toxic changes rats over the rats to the dose of 500 mg/kg and thus, found to be safe compounds with a lethal dose (LD_{50}) of at least 500 mg/kg when being administered via an oral route.

INDUSTRIAL APPLICABILITY

The present invention relates to antisense monomers and oligomers which can inhibit transcription for the production of disease-inducing proteins. The antisense monomers and oligomers of the present invention have higher binding affinity for RNA, the target of general antisense drugs, than that for DNA. In addition, they have increased nuclease resistance and improved permeability of cell membrane.

The monomers and oligomers of the present invention can be used for antisense therapy inhibiting the expression of genes inducing diseases, hybridization of gene cloning and reagents for diagnosis. They also provide useful tools for the investigation of proteins.

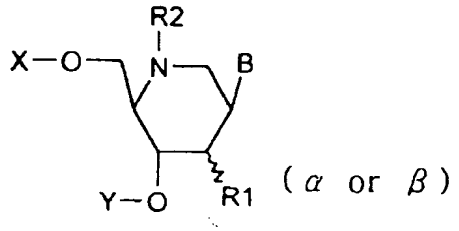
Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a

basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

What is Claimed is

1. Modified nucleotide monomers represented by the formula 1, in which five-membered ribose, sugar of natural nucleotide, is substituted with azasugar of six-membered ring.

Formula 1



(α or β)

Wherein

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl;

alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) X is hydrogen or hydroxy protecting group, and

(5) Y is hydrogen, phosphate, activated phosphate, activated phosphite or solid support.

2. The nucleotide monomers according to claim 1, wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

3. The nucleotide monomers according to claim 1, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl, fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

4. The nucleotide monomers according to claim 1, which are represented by the formula 1 and is selected from the group comprising:

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 1);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-

dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine (the compound of Example 2);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxyethoxypiperidine-3-yl}adenine (the compound of Example 3);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethylpiperidine-3-yl}adenine (the compound of Example 4);

6-N-benzoyl-{(3R,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 5);

6-N-benzoyl-{(3S,4S,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-ethoxypiperidine-3-yl} adenine (the compound of Example 6);

6-N-benzoyl-{(3R,4R,5R,6R)-N-methyl-5-[(2-cyano ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyl trityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 7);

6-N-benzoyl-{(3R,4R,5R,6R)-N-propyl-5-[(2-cyano ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyl trityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 8);

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzyl-5-[(2-cyano

ethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyl trityloxymethyl-4-methoxypiperidine-3-yl}adenine (the compound of Example 9);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-cyanobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 10);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-fluorobenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 11);

6-N-benzoyl-{(3R,4R,5R,6R)-N-(4-methoxybenzyl)-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} adenine (the compound of Example 12);

4-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} cytosine (the compound of Example 13);

2-N-isobutyryl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl} guanine (the compound of Example 14);

{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrithyl oxymethyl-4-methoxypiperidine-3-yl}thymine(the compound of Example 15);

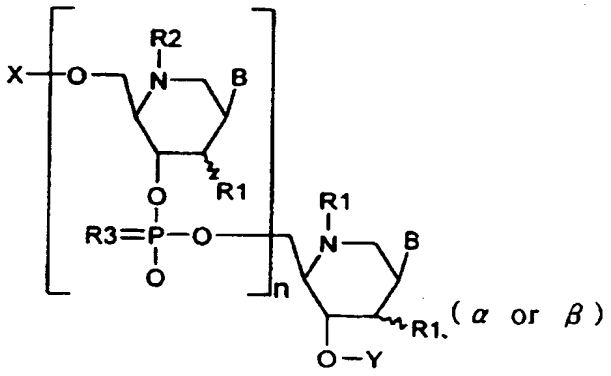
{(3R,4R,5R,6R)-N-methyl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine (the compound of Example 16);

{(3R,4R,5R,6R)-N-fluorenyl-5-[(2-cyanoethoxy)N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-methoxypiperidine-3-yl}thymine (the compound of Example 17); and

6-N-benzoyl-{(3R,4R,5R,6R)-N-benzhydryl-5-[(2-cyanoethoxy)(N,N-diisopropylamino)phosphinoxy]-6-dimethyltrityloxymethyl-4-isobutyryloxypiperidine-3-yl}adenine (the compound of Example 18);

5. Antisense oligomers represented by the formula 2, which are prepared with the nucleotide monomers of claim 1 as a part or whole of oligonucleotide.

Formula 2



Wherein,

n is 0 to 30,

(1) B is a natural nucleobase or a modified nucleobase with or without protecting group,

(2) R^1 is hydrogen; α- or β-hydroxy; α- or β-lower molecular alkoxy such as α- or β-methoxy, or α- or β-ethoxy; α- or β-methoxyethoxy; α- or β-halogen such as α- or β-fluoro; α- or β-aminoalkoxy such as α- or β-aminomethoxy or α- or β-aminoethoxy; α- or β-dimethylamino-oxyalkoxy such as α- or β-dimethylamino oxyethyloxy; or α- or β-O-acyl,

(3) R^2 is hydrogen; araalkyl such as benzyl, methylbenzyl, ethylbenzyl, dimethylbenzyl, diphenylmethyl or halodiphenylmethyl; nitrobenzyl; haloaraalkyl such as fluorobenzyl; cyanobenzyl; alcoxybenzyl such as methoxybenzyl or ethoxybenzyl; lower molecular alkyl such as methyl, ethyl, propyl or tertbutyl; aryl with or without substituent of phenyl or halophenyl; heterophenyl; heteroaryl; naphtaryl; or fluorenyl(Fmoc),

(4) R^3 is oxygen or sulfur,

(5) X is hydrogen or hydroxy protecting group, conjugate group or oligonucleotide, and

(6) Y is hydrogen, phophate, active phosphate, active phosphite, solid support, conjugate group or oligonucleotide.

6. The antisense oligomers according to claim 5,

wherein R1 is selected from the group consisting of hydrogen, methoxy, ethoxy and methoxyethoxy.

7. The antisense oligomers according to claim 5, wherein R2 is selected from the group consisting of diphenylmethyl, methyl, *t*-butyl, benzyl, cyanobenzyl, fluorobenzyl, methoxybenzyl and fluorenyl (Fmoc).

8. Chimeric oligomers, which have the monomers of claim 1 or the antisense oligomers of claim 5 at both ends of the molecules, and phosphodiesters or phosphothioate oligonucleotides in their middle.

9. A method for preparing nucleotide monomers of claim 1, which comprises the following steps:

(1) Obtaining a ketone compound through acidification of glucose with a protecting group;

(2) Removing the protecting group in the compound of step (1) using an acidic resin;

(3) Obtaining the azasugar of six-membered ring by ring formation of the compound of step (2);

(4) Protecting a primary alcohol group at position C-6 and secondary alcohol at position C-5 of the compound of step (3);

(5) Protecting a secondary alcohol at position C-3 of the compound of step (4), followed by alkylation of alcohol at position C-4;

(6) Removing and mesylating the protecting group at C-3 position of the compound of step (5);

(7) Preparing nucleosides by condensation of the compound of step (6) with base; and

(8) Linking phosphate groups to the nucleosides of (7).

10. A process for preparing antisense oligomers of claim 5, which comprises:

(1) Substituting dimethoxytrithyl group for a primary hydroxyl group linked to the sugar of a nucleotide monomer, phosphoramidite group for a secondary alcohol group, and to protect nucleobases except thymine, with an appropriate protecting group;

(2) Performing condensation reaction of the monomer of step (1) linked to solid support with a oligonucleotide;

(3) Removing the solid support and protecting group from the oligomer of step (2);

(4) Removing a 5'-hydroxy protecting group from the oligomer.

11. The process for preparing antisense oligomers according to claim 10, wherein condensation of step (2) is characterized by having the nucleotide monomer linked to the solid support at position 3'.

12. The process for preparing antisense oligomers according to claim 10, wherein the condensation of step (2) is characterized by having a nucleotide monomer linked to the solid support at positions except 3'-terminus, prepared via standard phoshoamidite process using a DNA synthesizer.

13. Pharmaceutical compositions containing the antisense oligomers of claim 5 or the chimeric oligomers of claim 8 as active ingredients, which are effective for inhibition or prevention of proteins syntheses.

14. Pharmaceutical compositions containing the antisense oligomers of claim 5 or the chimeric oligomers of claim 8 as active ingredients, which are effective for the treatment of hepatitis of viral or bacterial origin, cancers and immune diseases.

SEQUENCE LISTING

<110> DONGBU HANNONG CHEMICAL CO., LTD.

<120> Nucleotide monomer containing six-membered azasugar and antisense oligomer thereof

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/00713

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07H 21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
IPC7 C07H 21/00

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
STN caslink database structure search, IBM patent database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9932481 A1(Alcon Laboratories Inc.) 1 Jul. 1999 (7.01. 1999)	1-8
A	US 5623070 A (Isis Pharmaceuticals Inc.) 22 Apr. 1997 (4. 22. 1997)	1-8
A	US 5386023 A (Isis Pharmaceuticals Inc.) 31. Jan. 1995 (1. 01. 1995)	1-8
P	US 5965721 A (Isis Pharmaceuticals Inc.) 12. Oct. 1999 (10. 12. 1999)	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
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"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 NOVEMBER 2000 (22.11.2000)	28 NOVEMBER 2000 (28.11.2000)

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